Current Applied Science and Technology Vol. 21 No. 2 (April-June 2021)

Review article

Animal Biotechnology and Ethical Issues

Sanu Mahatthanadull[1]* and Dusanee Thanaboripat[2]

[1]International Buddhist Studies College, Mahachulalongkornrajvidayalaya University, Ayutthaya, Thailand
[2]King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Received: 9 September 2020, Revised: 27 November 2020, Accepted: 30 December 2020

Abstract

Animal biotechnology is one of the main areas of biotechnology which concerns the application of animals, including transgenic animals, in various fields. Even though these transgenic animals can be useful for improving the welfare of humans and animals, the well-being of animals being used in the studies may be negatively affected. Ethical issues relating to experimentation on animals and the production of transgenic animals are discussed in this review, and included here is a consideration of the phenomena of 'conditional ethical blindness'. Finding alternative protocols including the 4 Rs (reduction, refinement, replacement, and responsibility) to minimize the employment of animals in scientific procedures is one way to solve these problematic issues. These strategies can be successively utilized for certain animal biotechnological protocols and can be used to intelligently avoid unethical manipulation of animals.

Keywords: animal; animal biotechnology; transgenic animal; ethical issue; ethics
DOI...........

1. Introduction

Nowadays, the advancement of science and technology has become a fundamental part of human's world. Observation and experimentation are the most significant steps in the examination of phenomena. Scientific methodologies lead scientists to consider the truth of things that can only be proven by the 5 senses, i.e. sight-eye, smell-nose, taste-tongue, hearing-ear, and touch-body [1]. However, many scientists are exploring and using the Buddha's teaching to explain about scientific discovery, and there exists a close intellectual bond between Buddhism and modern science [2]. Vast areas of science and technology related to Buddhism are being investigated including quantum physics, biology, modern biotechnology and so on. Biotechnology is a multidisciplinary area which covers the studies of basic sciences such as biochemistry, genetics, microbiology, immunology, chemical engineering, molecular and cell biology, including human, plant and animal physiology.

*Corresponding author: Tel.: +66814079000
E-mail: petchsanu@gmail.com

Applied sciences such as animal biotechnology, plant biotechnology, food biotechnology, agricultural biotechnology, microbial biotechnology, industrial biotechnology, environmental biotechnology and pharmaceutics are also included under the rubric of biotechnology. Smith [3] has concluded that biotechnology implies the application of microorganisms, plant or animal cells, and enzymes to synthesize, transform or even breakdown materials. The areas of health care and medicine, followed by food and agricultural technology are the latest applications of biotechnology. Animal biotechnology is depicted as being one of the most important branches of biotechnology, and the development of animal biotechnology has contributed immensely to human health, nutrition and economy [4].

There are many definitions of "animal biotechnology", and these definitions reflect biotechnology novelty and established practice [5]. For example, it may be routine breeding technologies, e.g., artificial insemination, and some older breeding practices [6, 7]. Straughan [8] defined that "animal biotechnology" covered many well-documented procedures of conventional livestock breeding including the utilization of artificial insemination and performance testing. Animal biotechnology also includes vast developments in reproductive physiology like embryo transfer (surrogacy) and *in vitro* fertilization (test tube babies).

Animal biotechnology has been applied mainly for two purposes: to produce animals that are employed in fundamental biological research for biological development and function, and to create disease models that can mimic human diseases and thus be used for studying diseases (Parkinson's, cystic fibrosis, cancer, etc.) as well as for testing new drugs. The history of animal biotechnology began in the early 1980s with the production of genetically modified animals in which cloned sheep were created by embryonic cell transfer [9]. Somatic cell nuclear transfer was first used in 1996 to create the cloned sheep, Dolly [10]. Although the majority of work within animal biotechnology has been carried out using laboratory mice and sheep, as well as cattle, these technologies have been more recently adapted to other animal species like pigs, cats, goats, and horses. Methodologies and success rates vary from species to species [5]. For example, the success rate of using transgenic animals (pigs, sheep and cattle, and other animals) is only about 1% compared with 2-5% in mice [3].

Recombinant DNA technology (rDNA) is the employment of *in vitro* nucleic acid techniques including recombinant DNA and direct injection of nucleic acids into cells and organelles. It is also the technique that produces hybrid DNA by joining pieces of DNA from different organisms [4, 11]. The transferring of a foreign gene (for growth hormone) from a rat into a mouse by rDNA was the first reported example [11]. Cloning and genetic engineering (genetic modification) are two main techniques used to produce the desired organism. Cloning can be defined as the production of similar populations of genetically identical individuals whereas genetic engineering refers to the modification of DNA in order to create new types of organisms by inserting or deleting genes. Reproductive cloning is the re-creation of a whole organism, and recent advances in biotechnology have made it possible to reproductively clone mammals in the laboratory [12, 13].

1.1 The use of transgenic animals

Although there are some risks associated with the production of genetically modified animals or transgenic animals, such as the fact that an insertion of foreign gene may disturb the genome expression, and that normal reproduction may result in a transgene being released into a wild population, the benefits of cloning techniques are considerable, and some of the key benefits are as follows [14]:

1) Animals may be introduced with desired characteristics that require medical treatments or any feed supplements.

2) A desired characteristic of offspring might be established within one generation.

3) The characteristics needed can be chosen with greater accuracy and specificity.

Furthermore, transgenic animals can be produced to improve the quality of meat, the efficiency of meat and egg production, and the quality and quantity of milk and wool. In addition, transgenic animals may have more disease resistance and can be used for the production of low-cost pharmaceuticals and biologicals. A novel and commercially useful utilization of transgenic animals is the production of pharmaceuticals or human proteins in transgenic lactating animals [3]. Transgenic animals can become bioreactors, producing products like pharmaceuticals that were formerly produced only in a culture of transgenic microorganisms [15]. Singh *et al*. [4] added that the development of transgenic animals was one of the most important advances during the last decades. Transgenic livestock have played a vital role in the large-scale production of novel medications and pharmaceuticals including high valued food for human applications. Numerous recombinant proteins and other pharmaceuticals for humans and livestock therapeutic applications are being produced using transgenic animals [16].

Transgenic technology was originally developed as a research tool for the study of gene function in disease models. The first transgenic mouse was developed in the 1980s using pronuclear microinjection (a process known as transfection, which is a technique that makes use of finely constructed glass needles to inject purified DNA into the fertilized eggs of the selected species) of the exogenous DNA [3]. Moreover, genetic modifications must be achieved in cells if the yield is too low (low rate of random integration and targeted integration via homologous recombination) and this can be done by developing chimeric or transgenic animals [17].

A transgenic organism is an animal or plant that is generated by the introduction of a foreign gene (a transgene) or DNA and the transgene can change the characteristics (phenotype) of the organism [18]. Transgenic animals can be addressed as genetically engineered animals, or genetically manipulated, genetically modified, genetically altered animals, and biotechnology-derived animals. A genetically engineered animal is also defined as "an animal that has had an alteration in its nuclear or mitochondrial DNA (addition, substitution, or deletion of some part of animal's genetic material or insertion of foreign DNA) accomplished through a deliberate human technological intervention." In addition, transgenic animals may be animals that have undergone induced mutations, e.g., by radiation or chemicals which is different from mutations that naturally occur in populations. Cloned animals are also considered to be genetically engineered because there is a direct intervention and planning involved in producing these animals [19]. Transgenic animals have been widely used for various purposes but mainly for human health and animal production. Transgenic mammals are significantly used in the areas of biotechnological, agricultural, biological and biomedical sciences including the production of human gene therapy, antibody production, pharmaceuticals, and disease models for the development of new treatments, blood replacement and organ transfer from transgenic animals to humans [20, 21].

Animal models serve as models for understanding certain biological phenomena, with the prospect that discoveries made in organism models will render insights into the biological functions of other organisms [4, 18]. They are also useful for exploring the underlying mechanisms of physiology and disease in humans [18]. Animal models have been used to create new medicines for different diseases. Pig, for instance, shares several anatomical and physiological homologies with human and mimics the human situations in many ways more accurately than other species [22]. Thus, transgenic pigs can be used as an animal model of human diseases such as cancer, cardiovascular diseases, Alzheimer's disease, diabetes mellitus and cystic fibrosis [23]. Furthermore, several types of small mammal models have been developed for cardiovascular abnormalities that occur naturally or that are induced experimentally [24].

Among the mammalian experimental models, transgenic mice are routinely used as mammalian models. Transgenic mice or Omega mice were engineered to carry genes from roundworm that were capable of producing essential omega-3 and omega-6 fatty acids from saturated fats or carbohydrates. These transgenic mice are used as a new animal model in order to understand the impact of fat on human health [25]. Transgenic fish, birds (poultry) and mammals such as goats, cows, pigs, rabbits, and sheep have been developed as exogenous protein production systems [4]. In particular, preferred ungulates are pigs, sheep, goats, buffaloes, oxen, antelopes, horse, donkeys, deer, mule, elk, caribou, llama, camels and so on. However, working with larger animals, such as cattle, proved to be much more expensive than working with smaller animals such as mice, pigs, goat and sheep. Therefore, some work only focused on breed-early/lactate-early animals like pigs, sheep, and goats [26].

Innovative materials can also be generated by transgenic animals. For instance, transgenic silkworm with chimeric silkworm or spider silk properties has been created by a group of researchers. Another research group produced transgenic silkworms with the ability to produce fluorescent silk by introducing genes (derived from a jellyfish) that coded for green fluorescent protein [27]. In addition, transgenic silkworm that could spin out spider silk, which is a super-tough fiber, was also engineered at Kraig Biocraft Laboratories (KBL), USA. Dragon silk, one of the latest products of this company, was produced from the fibers of these cocoons. This silk is stronger than steel and lighter, tougher, and much more flexible than Kevlar but Kevlar has slightly lower tensile strength than this synthetic fiber. The company is also searching for possible uses of this silk in defensive clothing and other equipment [28].

Recently, rhesus macaques (*Macaca mulatta*), the most widely used monkey model, have been tested for vaccine trial against COVID-19 by injecting a weakened respiratory virus coding of SARS-CoV-2's spike protein in 32 rhesus macaques [29]. Moreover, researchers from Sinovac Biotech, a private Beijing-based company, also introduced two different doses of their COVID-19 vaccine to 8 rhesus macaques. After 3 weeks, SARS-CoV-2, the virus that causes COVID-19, was then introduced into the monkeys' lungs through tubes down their tracheas, and it was found that no monkey developed a full-blown infection [30].

1.2 Ethical issues in animal biotechnology

The use of animals in experimental research has increased due to the advancement of research and development in medical technology. Each year, millions of animals are being experimentally used worldwide and 90% of these animals are mice and rats, including cats, dogs, rabbits and primates [31]. The pain, distress and even death of animals during the experimentation have been a dispute issue for years [32].

Monkeys are one of the most controversial animal models used in experiments because they have a close genetic relationship to humans. However, less than 1% of animals used in European countries (EU) are monkeys. Monkeys are used only if it is not possible to use a non-animal model, or other animal species for the research being undertaken. However, monkeys are frequently used for testing Covid-19 treatments due to their similarity to humans and they are valuable as they provide the most reliable source of information [33].

Modern biotechnology, especially animal biotechnology, has the possibility to draw a wide range of "moral and ethical concerns". This is probably because of basic disagreements about what our behavior and attitudes towards animals ought to be [8]. Definitions of morals and ethics can vary with country, culture, religion, and so on. Different cultures have different moral codes, and many of the practices and attitudes that we may regard as correct or natural are really only cultural products [34]. However, it is debated that the majority of human beings seems to be content that they could benefit from the use of animals, either directly or indirectly. Therefore, the simple use of animals by human beings has not normally been seen as a matter for moral concern [8]. Many different ethical problems concerning cloning and transgenesis depend on people's beliefs. Recently, the number of animals derived from genetic engineering technology has increased significantly, and ethical issues related to this kind of science and animal welfare have grown [35].

Ethical codes, which are sets of principles for human beings to follow, have been created by a number of professional organizations specifically for their respective fields. In Thailand, the Animals for Scientific Purposes Act 2015 (2558 B.E.) has been established with the aim to protect animals, that are used in any research and other scientific procedures, from unnecessary pain, distress, suffering, and lasting harm [36]. Thus, decisions on animal experimentation have often been taken not on the basis of established science-based evidence of environmental risk but rather on ethical grounds.

Singer [37] described "conditional ethical blindness" in terms of a researcher in the area of animal experimentation, who, conditioned by professional rewards, ignores the ethical issues raised by the experiments just like a rat that is conditioned to press a lever in return for a reward of food. It is not only the experimenters themselves who suffer from conditioned ethical blindness but also research institutes can sometimes have conditional ethical blindness. Some researchers who work with animals may want to help solve the problems of human aggression, however, he or she can later find that rewards or professional prestige are more important than ethics.

Lopez [2] claimed that Buddhism is, in fact, the scientific religion best suited for modernity throughout the world. Thus, Buddhism remains significant and necessary for humans [38]. According to Buddhism, humans have to develop wisdom and virtue at the same time. If science goes together with virtue, it will provide more usefulness and convenience to humans.

Transgenic animals arising from intended genetic change can violate animals and sometimes lead to crippling and even death. Thus, the application of genetic modification animals as the models of human disease may cause animals to suffer as they develop the disease [39]. In Buddhism, it is morally wrong to cause harm to animals. An understanding of the Buddhist concept of *kamma* can be very useful for encouraging humans to be more responsible and to improve their moral standards as well as refraining from unwholesome actions [40]. The Buddhist way may help solve problems in a manner that gives the least damaging to all the people involved [41].

Animals are sentient creatures, and they often value their family. On the one hand, human beings are animals; on the other hand, humans define themselves in opposition to all other animals. Through this dualistic interplay, animality has become a fruitful resource for defining what it means to be human [42]. Some people may be against the utilization of animals by humans for any purpose, whereas others may have special concerns about the impacts of genetic engineering and cloning that might affect animal health and welfare. Some people may find it upsetting that industrial terminology like "transgenic animal bioreactors" is used to describe genetically engineered animals that produce human therapeutic or industrial proteins or agricultural products such as food or even as disease model. Mahatthanadull [43] argued against the creation of genetically modified organisms that it intentionally conveys contamination of "alien genes" into the immaculate environment pervasively. Directionless mutation may ultimately affect the biodiversity of the ecosystem in a way that is beyond human control. From this aspect, the ecological equilibrium could be destroyed by just a few scientific equations. A new overwhelming species will infiltrate the original species, and in doing so causing the extinction of the original species. However, animal cloning and transgenic methodologies can surely create welfare concerns. There is always the disturbing possibility that as success rates in animal transgenics get higher, there will be increased demand for the use of animals in this way. Thus, effective and responsible communication among scientific community, industry, and government stakeholders are vital in order to reach a consensus on the acceptability levels of risk for products of animal biotechnology, as well as to identify which set of values should be applied to certain acceptable uses of animal biotechnology [44].

The use of animals in animal biotechnology can cause animals to suffer, therefore, we have a moral obligation to protect them from suffering in every way we can [31]. Based on Buddhism, the killing or harming of all living beings including animals could violate the first precept. To abstain from injury to living creatures is the right action for moral conduct [45]. Mahatthanadull and

Mahatthanadull [46] added that the "penalties for the violation of the Five Precepts" are important as an aim to raise awareness of the consequences of negligently transgressing the precepts. For example, the killing of a small insect such as an ant attracts a lesser penalty than killing a larger animal such as bird or reptile species. The killing of larger animal is a weightier act because it requires a greater degree of intention and force than does the killing of a smaller one, i.e. the larger the animal, the greater the penalty. In fact, the Buddhist commentaries mention four factors that determine the severity of ill-effects that result from killing various kinds of creatures, namely: the degree of virtue (or value) of the animal killed, the size of the animal, the effort made to kill, and the intention with which the killing is done [45]. Therefore, the Buddha makes it clear that the mistreating or killing of larger and more developed animals should be avoided and furthermore that the research-related treatment of animals must be done with well-thought-out and good and upright intention.

According to scientist, all life is classified by hierarchical levels of organization and the cell holds a special place in the hierarchy of life because it is the lowest level of living organisms. Nervous systems distinguish animals from all other kingdoms of life and certain properties of human brains distinguish humans from all other animals. The sizes and shapes of animals affect animals' interactions with external environments. A large animal, such as an elephant, has very different body proportions than a small animal, such as mouse [47] and the perceptual abilities of various animal types (in terms of their senses and brains) vary according to the animal classification. Thus, the physical size of animals and *kamma* are related according to the animal classification designed by scientist [48].

1.3 Alternative methods to animal testing

Technologies related to transgenic and cloned animals might be ethically unacceptable if the animals suffer or are frustrated to a greater degree than that experienced by normal animals of the same species under similar husbandry. However, if there are no adverse effects on individual animals, there is no basis for ethical objections to animal biotechnology. The use of transgenic animals is facing concern of negative impacts on environment because of disrupting ecological balance, food safety concern, ethical and religious concerns. Thus, recommendations by both government and commercial entities are made by supporting of research that helps develop promising bio-therapeutics and biotechnology products. Information and knowledge regarding genetic engineering, production methods, products and regulatory processes should be made available to the public. Education concerning the advantages and challenges related to animal biotechnology is the key to public understanding. In addition, government inadvertence, regulations as well as stringent rules on the research of genetic engineering and transgenic animals should also be considered [49].

In order to overcome the drawbacks of animal experimentation and to avoid unethical procedures, alternatives to animal testing have been proposed [50]. The strategy of 3 Rs, i.e. the reduction, refinement and replacement of laboratory use of animals, can be applied [51]. Three Rs was first described in 1959 by Russell and Burch as the benevolent and ethical way to use animals in research testing and their work was followed worldwide with the establishment of many other research tests [52]. These approaches suggest some ways to make animal experiments more humane by minimizing the numbers of animals so that the numbers of animals used in experiments are reduced, that is by 'reduction'. At the same time the use of animals has to be planned and refined carefully in a way that pain and distress caused during the experiment should be lessen, and this constitutes 'refinement'. For 'replacement', it is described as the use of simpler life forms such as lower vertebrates and invertebrates or the use of non-sentient materials instead of living conscious vertebrates. Two kinds of replacements, i.e. relative and absolute replacement can be applied [53].

These methods can provide an alternative means for drug and chemical testing, up to certain levels. The advantages of these methods are time efficiency, require less man power, and these methods are also cost effectiveness. Doke and Dhawale [32] also suggested alternative methods as follows:

1) *Computer models*: Computers can be used to generate simulations for the prediction of many possibilities of biological and toxic effects of a chemical or potential drug candidate without dissecting animals.

2) *Cells and tissue cultures*: *In vitro* cell and tissue cultures involving the growth of cells in laboratory environment can be used as a considerable alternative for animal experiments. The cells and tissues from animals like brain, kidney, liver, skin can be removed and grown outside the body in a suitable medium for further use. These methodologies are also routinely applied for the preliminary screening of potential drug molecules or chemicals in order to check their efficacy and toxicity [54]. The benefits of these techniques are easy to follow, less time consuming and less expensive when compared to animal tests.

3) *Alternative organisms*: In order to replace experimental animals, different model organisms can be used, e.g., lower vertebrates (zebra fish, *Danio rerio*), invertebrates (fruit fly, *Drosophila melanogaster*, nematode, *Caenorhabditis elegans*) and microorganisms (yeast, *Saccharomyces cerevisiae*).

Because of the advancement and rapid development in biomedical sciences, 'responsibility' is the 4^{th} R added to the basic 3Rs. The addition of 'responsibility' is to let people realize the importance of integrity and honesty in the reasonable and proper use of animals in laboratory because an issue of animal welfare is as important as human welfare [55]. In addition, advanced or sophisticated technologies that are used to replace live animals also include cell-based tests (in vitro); tests that use tissue taken from dead animals or humans (ex vivo); computer-based modelling (in silico); chemical-based analytical tests (in chemico) as well as ethical human studies (in vivo) [56]. However, these alternatives cannot completely eliminate the necessity for the use of animals in the laboratory because intact animals can provide a better model of the complex interaction regarding the physiological process than other alternative techniques [52]. Thus, in order to motivate science to change its paradigm, policies have to be targeted and amplified to reduce animal experimentation as well as to lift regulatory malaise and to provide more funding for development of new approaches [56].

Therefore, these alternative protocols of the 3 Rs plus responsibility can provide dependable outcomes and minimize the use of animals in scientific procedures. Although these strategies can also minimize or in some case nullify the need for ethically-questionable animal experimentation, some studies may still require the use of animals.

2. Conclusions

Animal biotechnology has become an important branch of biotechnology and it is being used in an increasing number of applications in basic science, medical science and agriculture. The core of animal biotechnology is addressed on transgenic animals produced through cloning and genetic engineering techniques which are nowadays becoming extremely advanced and common. There seems to be little doubt that the use of animal biotechnology benefits human welfare. However, whilst using animals as a model, scientific risk assessment and use of the precautionary principle, as well as ethical guidelines have to be highly concerned. Some of the ethical issues are still questionable and responses to them may well depend on individual's opinion, culture and religion. 'Conditional ethical blindness' is also a factor that can determine how individuals, organizations and society see and respond to the main ethical issues. Therefore, one way to solve these problems is the use of alternative protocols such as reduction, refinement, replacement and responsibility to minimize the use of animals as well as to avoid animal ethical controversies. When experimentation with animals cannot be avoided, they must be treated with as much kindness and compassion as possible. Effective and responsible communication through scientific community, industry and government stakeholders are also required in order to reach a consensus of society on the acceptance of genetic engineering and animal cloning.

References

[1] Phra Dharmakosajarn, 1998. Educating for balance: a Buddhist perspective. *19th General Conference of the World Council for Curriculum and Instruction*, Bangkok, Thailand, July 14, 1998.

[2] Lopez, D.S., 2008. *Buddhism & Science. A Guide for a Perplex*. Chicago and London: The University of Chicago Press.

[3] Smith, J.E., 2009. *Biotechnology*. 5th ed. Cambridge: Cambridge University Press.

[4] Singh, B., Gautam, S.K., Chauhan, M.S. and Singla, S.K., 2015. *Animal Biotechnology*. New Delhi: The Energy and Resources Institute (TERI).

[5] Gjerris, M., Olsson, A. and Sandøe, P., 2006. Animal biotechnology and animal welfare. In: *Ethical Eye: Animal Welfare*. Strasbourg: Council of Europe, pp. 89-110.

[6] American Israeli Cooperative Enterprise (AICE), 2019. *Breakthrough Dividend*. [online] Available at: www.jewishvirtuallibrary.org/jsource/ biotech/ bdcontent.html

[7] National Academy ofSciences (NAS), 2002. *Animal Biotechnology: Science Concerns*. Washington D.C.: National Academy of Science.

[8] Straughan, R., 1999. *Ethics, Morality and Animal Biotechnology*. Swindon: The Biotechnology and Biological Sciences Research Council.

[9] Willadsen, S.M., 1986. Nuclear transplantation in sheep embryos. *Nature*, 320, 63-65.

[10] Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H., 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature*, 385, 810-813.

[11] Smith, J.E., 2004. *Biotechnology*. 4th ed. Cambridge: Cambridge University Press.

[12] Molnar, C. and Gair, J., 2019. *10.1 Cloning and Genetic Engineering*. [online] Available at: https://pressbooks.bccampus.ca/conceptsofbiologymolnarcamosun/chapter/10-1cloning-and-genetic-engineering/.

[13] Twine, R., 2010. *Animal as Biotechnology. Ethics, Sustainability and Critical Animal Studies.* New York: Earthscan.

[14] Singhal, M. and Kansara, N., 2010. Transgenic animals: production and application. *International Journal of Pharmaceutical Sciences and Research*, 1(9), 12-22.

[15] Smith, J.E., 2004. *Biotechnology*. 4th ed. Cambridge: Cambridge University Press.

[16] Houdbine, L.M., 2009. Production of pharmaceutical proteins by transgenic animals, *Comparative Immunology Microbiology and Infectious Diseases*, 32, 1393-1398.

[17] Laible, G., 2018. Production of transgenic livestock: Overview of transgenic technologies. In: H. Niemann and C. Wrenzycki, eds. *Animal Biotechnology 2. Emerging Breeding Technologies.* Cham: Springer, pp. 95-121.

[18] Weitzman, J. and Weitzman, M., 2017. *30-Second Genetics*. London: Ivy Press

[19] Ormandy, E.H., Dale, J. and Griffin, G., 2011. Genetic engineering of animals: Ethical issues, including welfare concerns. *Canadian Veterinary Journal*, 52(5), 544-550.

[20] Wall, R.J. and Seidel, G.E., 1992. Transgenic farm animals, a critical analysis. *Theriogenology*, 38(2), 337-357.

[21] Houdebine, L.-M., 2005. Use of transgenic animals to improve human health and animal production. *Reproduction in Domestic Animals*, 40(4), 269-281.

[22] Rogers, C.S., Abraham, W.M., Brogden, K.A., Engelhardt, J.F., Fisher, J.T., McCray, P.B., McLennan, G., Meyerholz, D.K., Namati, E., Ostedgaard, L.S., Prather, R.S., Sabater, J.R., Stoltz, D.A., Zabner, J. and Welsh, M.J., 2008. The porcine lung as a potential model for cystic fibrosis. *American Journal of Physiology-Lung Cellular and Molecular Physiology,* 295, L240-L263.
[23] Aigner, B.S., Renner, S., Kessler, B., Klymiuk, N., Kurome, M. and Wünsch, A., 2010. Transgenic pigs as models for translational biomedical research. *Journal of Molecular Medicine,* 88(7), 653-664.
[24] Roths, J.B., Foxworth, W.B., McArthur, M.J., Montgomery, C.A. and Kier, A.B., 1999. Spontaneous and engineered mutant mice as models for experimental and comparative pathology: History, comparison, and developmental technology. *Laboratory Animal Science,* 49, 2-34.
[25] Pai, V.J., Wang, B., Li, X., Wu, L. and Kang, J.X., 2018. Transgenic mice convert carbohydrates to essential fatty acids. In: Sarah Lombard, ed. *Animal Biotechnology*. New York: Callisto Reference, pp. 1-6.
[26] Clark, A.J., Ali, S., Archibald, A.L., Bessos, H., Brown, P., Harris, S., McClenaghan, M., Prowse, C., Simons, J.P. and Whitelaw, C.B., 1989. The molecular manipulation of milk composition. *Genome,* 31, 950-955.
[27] Clark, D.P. and Pazdernik, N.J., 2016. Transgenic animals. In: *Biotechnology,* 2nd ed. Amsterdam: Elsevier, pp. 493-521.
[28] Offord, C., 2017. *Meet the Transgenic Silkworms That Are Spinning Out Spider Silk*. [online] Available at: https://www.the-scientist.com/notebook/meet-the-transgenic-silkworms-that-are-spinning-out-spider-silk-30212.
[29] Mercado, N.B., Zahn, R., Wegmann, F., Loos, C., Chandrashekar, A., Yu, J., Liu, J., Peter, L., McMahan, K., Tostanoski, L.H., He, X., Martinez, D.R., Rutten, L., Bos, R., van Manen, D., Vellinga, J., Custers, J., Langedijk, J.P., Kwaks, T., Bakkers, M.J.G., Zuijdgeest, D., Huber, S.K.R., Atyeo, C., Fischinger, S., Burke, J.S., Feldman, J., Hauser, B.M., Caradonna, T.M., Bondzie, E.A., Dagotto, G., Gebre, M.S., Hoffman, E., Jacob-Dolan, C., Kirilova, M., Li, Z., Lin, Z., Mahrokhian, S.H., Maxfield, L.F., Nampanya, F., Nityanandam, R., Nkolola, J.P., Patel, S., Ventura, J.D., Verrington, K., Wan, H., Pessaint, L., Ry, A.V., Blade, K., Strasbaugh, A., Cabus, M., Brown, R., Cook, A., Zouantchangadou, S., Teow, E., Anderson, H., Lewis, M.G., Cai, Y., Vhen, B., Schmidt, A.G., Reeves, R.K., Baric, R.S., Lauffenburger, D.A., Alter, G., Stoffels, P., Mammen, M., Hoof, J.V., Schuitemaker, H. and Barouch, D.H., 2020. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* (2020), https://doi.org/10.1038/s41586-020-2607-z.
[30] Cohen, J., 2020. *COVID-19 Vaccine Protects Monkeys from New Coronavirus, Chinese Biotech Reports*. [online] Available at: https://www.sciencemag.org/news/2020/04/covid-19-vaccine-protects-monkeys-new-coronavirus-chinese-biotech-reports.
[31] Phelps, N., 2004. *The Great Compassion. Buddhism and Animal Rights*. New York: Lantern Books.
[32] Doke, S.K. and Dhawale, S.C., 2015. Alternatives to animal testing: A review. *Saudi Pharmaceutical Journal,* 23, 223-229.
[33] European Animal Research Association, 2020. *Covid-19 Research Using Monkeys*. [online] Available at: https://www.eara.eu/post/covid-19-research-using-monkeys.
[34] Rachels, J., 2003. *The Elements of Moral Philosophy*. Boston: McGraw-Hill.
[35] Camera, D., Dimitrova, Ir., Doynova, M., Jachacz, L., Kachakova, D., Kepka, M., Ould Isselmou, C.B., Vornier, J.P. and Yungarva., Tsv., 2008. *Transgenic and Cloned Animals: Ethical Problem?* Perugia: EU Socrates Erasmus European Community.

[36] Kunjara, P., 2015. Animals for scientific purposes act 2015 (2558 B.E.). *Proceedings of the 14th Chulalongkorn University Veterinary Conference CUVC: Responsible for Lives*. Bangkok, Thailand, April 20-22, 2015, 75.

[37] Singer, P., 2002. *Animal Liberation. The Definitive Classic of the Animal Movement*. New York: HarperCollins Publishers.

[38] Surasith, N., 2016. *Tipitaka Studies*. Translated by Veerakarn Kanokkamalade. Ayutthaya: Mahachulaongkornrajavidayalaya Press.

[39] Brunk, C.G., Hartley, S., 2013. *Designer Animals. Mapping the Issues in Animal Biotechnology*. Toronto: University of Toronto Press.

[40] Ubeysekara, A., 2019. *The Concept of Kamma in Theravada Buddhism*. [online] Available at: https://drarisworld.wordpress.com/2016/09/25/the-concept-of-kamma-in-theravada-buddhism/.

[41] Taniguchi, S., 2019. *Biomedical Ethics. From a Buddhist Perspective*. [online] Available at: https://enlight.lib.ntu.edu.tw/FULLTEXT/JR-MAG/mag133029.pdf.

[42] Ohnuma, R., 2017. *Unfortunate Destiny. Animals in the Indian Buddhist Imagination*. Oxford: Oxford University Press.

[43] Mahatthanadull, S., 2015. Buddhist response to environmental degradation under conceptual framework of the Five Niyāma. *Proceedings of the 1st MCU International Academic Conference (MIAC) on Buddhism and World Crisis*, Graduate School, MCU, Thailand, May 29, 2015, 65-78.

[44] Van Eenennaam, A.L., 2006. What is the future of animal biotechnology? *California Agriculture*, 60(3), 132-139.

[45] Payutto, P.A., 2017. *Buddhadhamma. The Laws of Nature and Their Benefits to Life*. 2nd ed. Translated by R. P. Moore. Bangkok: Buddhadhamma Foundation.

[46] Mahatthanadull, S. and Mahatthanadull, S., 2018. The five precepts: Criteria and the promotion of individual and social peace *JIABU*, 11(3), 180-193.

[47] Cambell, N.A., 1996. *Biology*. 4th ed. Menlo Park: The Benjamin/Cummings Publishing Company, Inc.

[48] Mahatthanadull, S. and Mahatthanadull, S., 2015. *Research Report on the Five Precepts: Criteria and the Promotion of Individual and Social Peace*. International Buddhist Studies College: Mahachulalongkornrajavidyalaya University.

[49] Rollin, B., 1996. *The Frankenstein Syndrome: Ethical and Social Issues in the Genetic Engineering of Animals*. Cambridge: Cambridge University Press.

[50] Doke, S.K. and Dhawale, S.C., 2015. Alternatives to animal testing: a review. *Saudi Pharmaceutical Journal*, 23(3), 223-229.

[51] Ranganatha, N. and Kuppast, I.J., 2012. A review on alternatives to animal testing methods in drug development. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4, 28-32.

[52] Mushtaq, S., Das, Y.K. and Aksoy, A., 2018. Alternative methods to animal experiments. *Turkiye Klinikleri Journal of Medical Sciences*, 38(2), 161-170.

[53] Balls, M., 1994. Replacement of animal procedures: Alternatives in research, education and testing. *Laboratory Animals*, 28(3), 193-211.

[54] Xu, K.-P., Li, X.-F. and Yu, F.-S.X., 2000. Corneal organ culture model for assessing epithelial responses to surfactants. *Toxicological Science*, 58(2), 306-314.

[55] Arora, T., Mehta, A.K., Joshi, V., Mehta, K.D., Rathor, N., Mediratta, P.K. and Sharma, K.K., 2011. Substitute of animals in drug research: An approach towards fulfilment of 4R's". *Indian Journal of Pharmaceutical Sciences*, 73(1), 1-6.

[56] Taylor, K., 2019. Recent Developments in Alternatives to Animal Testing. [online] Available at: https://www.wellbeingintlstudiesrepository.org/cgi/viewcontent.cgi?article=1005&context=appamet.