Editorial

New Techniques for Producing Transgenic Animals — a Mixed Blessing from Both the Scientific and Animal Welfare Perspectives

Coenraad Hendriksen and Horst Spielmann

A further increase in the use of genetically-modified animals will be against the European Union policy to move away from the use of animals in biomedical research

Recombinant DNA technology has provided the tools to modify the genome of an animal, either by knocking out genes (knock-out) or by inserting new genes (knock-in) that are linked to specific pathways or diseases. Genetic modification has had a major impact on biomedical research, by expanding the range of animal models available, and consequently, by increasing the numbers of animals that are used in research and testing. As a result, the trend of a reduction of experimental animal numbers that was very obvious in the 1970s and 1980s, came to a halt in the early 1990s. The subsequent increase in animal numbers is almost entirely due to the use of genetically-modified (GM) animals, in both basic and applied research, e.g. in drug development.

It is indisputable that research using GM animals has significantly contributed to progress in our understanding of (patho-)physiological pathways and disease mechanisms. However, that progress should not be overestimated. The integration of human transgenes into the mouse genome, 'humanises' the animal to some extent, but doesn't transform the animal into a miniature human being. Moreover, the claim that applying recombinant DNA technology contributes to the Three Rs must be challenged. A GM model may indeed be more relevant, so a study consequently requires a reduced number of animals compared to wild-type animals. However, the production and breeding of a GM line is based on complex procedures, and requires a high number of animals, including embryo donors, vasectomised males and foster females. In addition, the first litters have to be checked for phenotypic stability, and they are culled afterwards, while extensive backcrossing might be needed to transfer the transgene into the desired strain. Furthermore, the generation of a homozygous genetic background requires the cross-breeding of heterozygous animals, with the result that only one in four animals have the correct genotype for the experiment. Therefore, 'surplus' animals that are not used in the experiment should correctly be referred to as 'animals bred, but not used', which are usually euthanised.

This is illustrated by figures from The Netherlands,[1] which show that the number of animals being bred, but not used, almost equals the number of animals being used in experiments (579,338 used in experiments and 524,735 bred but not used). This number of surplus animals has increased significantly in the last decade, due to the breeding of GM animals. The moral status of these animals was discussed at an international workshop on *Animals bred, but not used in experiments*, held on 18–20 October 2013, in Santpoort, The Netherlands. It was concluded that, although these animals are not subjected to experimental procedures and are euthanised early in life after genotyping, they should be the subjects of moral concern, simply because their integrity is compromised.

Recombinant DNA technology is, by nature, highly innovative. In 2013, a new technique was published, which permits the development of a new GM line by inducing homozygous offspring in one procedural step. This technique, the CRISPR/Cas-9 system, is discussed in the current issue of *ATLA* by Combes and Balls.[2] Its use will significantly influence the category of animals 'bred, but not used', both positively and negatively, though the net result is still unclear. It is a positive perspective that the CRISPR/Cas-9 technique will reduce the number of 'bred but not used' animals per generated GM line. It makes possible the generation of stable lines without the need for extensive backcrossing into the desired strain. Thus, for

animal welfare reasons, the new method should be used to reduce animal suffering and the number of surplus animals produced. The other side of the coin, however, is that a significantly higher number of GM mouse lines will be produced, while overall animal numbers will not be reduced. In addition, the CRISPR/Cas-9 system can be applied in more animal species in addition to mice and fish, to which the conventional GM technologies were mostly restricted.

As discussed by Combes and Balls, the successful generation of the first GM non-human primate was recently published, and reports on successful genetic modification of other species are to be expected in the future. Thus, studies previously performed in GM mouse lines will be repeated in GM rats and other GM species and, in the end, significantly more GM animal lines will be produced in a wide range of species. Such an increase will definitely be against the European Union policy to move away from the use of animals in biomedical research, as expressed in *Council Directive 2010/63/EU*.[3]

Professor Coenraad Hendriksen
Faculty of Veterinary Medicine
Utrecht University
3508TD Utrecht
The Netherlands
E-mail: C.F.M.Hendriksen1@uu.nl

Professor Horst Spielmann
Institute of Pharmacy
Freie Universität Berlin
14195 Berlin
Germany
E-mail: horst.spielmann@fu-berlin.de

References

[1] Anon. (2013). *2012 Annual Review of the Dutch Food and Consumer Product Safety Authority on Animal Testing and Laboratory Animals*, 51pp. Utrecht, The Netherlands: Nederlandse Voedsel- en Warenautoriteit. Available at: http://www.rijksoverheid.nl/documenten-en-publicaties/jaarverslagen/2014/02/04/zo-doende-2012.html (Accessed 15.04.14).

[2] Combes, R.D. & Balls, M. (2014). Every silver lining has a cloud: The scientific and animal welfare issues surrounding new approaches to the production of transgenic animals. *ATLA* **42**, 137–145.

[3] Anon. (2010). *Directive 2010/63/EU* of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union* **L276**, 20.10.2010, 33–79.