

RSB Response to DEFRA Animal Welfare Committee call for information on the “Review on welfare implications of breeding and breeding technologies in commercial livestock agriculture”

March 2024

The Royal Society of Biology (RSB) is a single unified voice, representing a diverse membership of individuals, learned societies and other organisations. Our world-leading biosciences sector contributes strongly to the economy, and to society. We are committed to ensuring that we provide Government and other policymakers, including funders of biological education and research, with a distinct point of access to authoritative, independent, and evidence-based opinion, representative of the widest range of bioscience disciplines.

*The RSB welcomes the invitation to take part in the DEFRA Animal Welfare Committee¹'s call for evidence on the **impacts, opportunities and risks associated with selective breeding and breeding technologies used in the production of food animals**, part of the committee's ongoing review of the 2012 FAWC opinion on the welfare implications of breeding and breeding technologies in commercial livestock agriculture². We are pleased to provide comments informed by our membership of individuals and organisations with expert interests across the biosciences³.*

*In our response we have focused primarily on the **developments of genetic technologies** and their importance for **animal breeding, health and welfare**. This response is informed by a longstanding engagement with our members, expert committees, such as our Animal Science Group⁴ and Genome Editing Advisory Group, and a body of evidence collected during knowledge exchange events, such as our Animal Science Meeting series⁵. A report of our last Animal Science Meeting 2023 is included at the end of this document and the outputs of the roundtable discussions are relevant to this call for evidence. We have also engaged extensively with*

¹ <https://www.gov.uk/government/groups/animal-welfare-committee-awc>

² <https://www.gov.uk/government/publications/fawc-opinion-on-the-welfare-implications-of-breeding-and-breeding-technologies-in-commercial-livestock-agriculture>

³ <https://www.rsb.org.uk/membership>

⁴ <https://www.rsb.org.uk/policy/groups-and-committees/asg>

⁵ <https://www.rsb.org.uk/policy/groups-and-committees/asg/asg-membership/animal-science-meetings>

Parliament and the Government during the passage of the Genetic Technologies (Precision Breeding) Act 2023 (GT(PB)A 2023).

Key points and recommendations

1. In order to protect ecosystems and reduce biodiversity loss, agriculture must do more with less, while adapting to rising global demand, changing climate and emerging biosecurity risks. Novel breeding technologies and balanced breeding strategies can contribute to the realisation of positive outcomes for the farming sector, including enhanced animal health and welfare, for both terrestrial and aquatic livestock species.
2. Responsible and ethical biological innovation is key to achieving these goals. Innovative breeding approaches will emerge from the combination of genomic sciences, objective phenotyping, statistical and computation methodologies and advanced reproductive techniques. We provide examples in our response of how these disciplines can contribute knowledge and innovation for the benefit of humans and animals, but the application of such knowledge must be accompanied by the careful assessment and management of any emerging risks.
3. Genetic technologies have the potential to accelerate the pace of genetic improvement of livestock species - allowing much needed fast adaptations to emerging threats – particularly through the rational design of novel alleles, and the introgression and promotion of favourable alleles in more efficient ways than would be feasible using conventional breeding methods.
4. Infectious disease resistance is a major opportunity for application of novel breeding methods and could result in major animal welfare benefits. Genome editing approaches present similar challenges to other disease control strategies, such as the use of drugs or vaccination. However, as evident in the case of zoonotic pathogens with pandemic potential, a carefully thought-through regulatory approval pathway from proof of concept stage to commercial release, via progressive stages of biocontainment, intermediate small scale releases and longer term monitoring/surveillance of emerging impacts will be required. A One-Health approach is recommended.
5. The goals of balanced breeding programmes must include and prioritise animal health and welfare, sustainability, the safeguarding of genetic diversity, food safety and public health, alongside productivity.
6. In order for novel breeding methods to deliver enhanced animal welfare, an appropriate framework for lifetime animal welfare impact assessment is needed. The right choice of animal welfare indicators, which will include behavioural correlates of mental states, is necessary to build an overall measure of an animal's life experience and determine standards of good life and acceptable levels of animal welfare. The impacts of traits and breeding technologies on individual animals and between groups/breeds will need to be tested first in controlled, experimental conditions, then in bigger experimental herds by breeders and developers, but critically also in real-life farm conditions, where environmental conditions vary greatly.

7. A synergy between Precision Livestock Farming tools and Animal Welfare Assessment systems is needed to realise this last ambition. Tools for monitoring animal welfare indicators on commercial farms need to be co-designed and developed with the end-users, namely, farmers and those tasked with the husbandry of the animals. There is need to upskill and provide specific training to the farming community, if animal welfare is to be achieved on the ground. Technological interventions should be locally adapted to the needs and contexts of human and animal populations and their environments.
8. Animal welfare assessment and relative indicators will likely need to be bespoke to each trait or species. There should be both hypothesis-driven testing (to be determined on a case-by-case basis) and holistic assessment of basic welfare variables. Regulations of precision bred animals should aim to reach a level playing field across jurisdictions and proportionality, with respect to how current breeding processes are regulated.
9. Improved transparency and accessibility of breeding data, particularly those related to animal welfare indicators and collected on experimental and commercial farms, is necessary to enable animal welfare scientists and regulators to independently assess the delivery of higher animal welfare. New regulations under the GT(PB)A 2023 could support this outcome, while giving due regard to intellectual property and commercially sensitive information.
10. Regulation of novel breeding methods should be proportionate and enable innovation, while also realising the policy objectives and protection goals of a resilient food system that supports our societies, meets animal welfare needs, alongside preserving natural resources and ecosystems. Ideally, advances in knowledge and technological applications would best be communicated by animal scientists and breeders directly to regulators and policymakers through stakeholder fora, where upcoming policy/guidance documents are co-developed and tested with the regulated sector. The RSB Animal Science Group would welcome the opportunity to engage with DEFRA and the Animal Welfare Committee in follow-up conversations about criteria and approaches for animal welfare assessment of novel breeding technologies.

Questions from the call for information

Section 1

1. Please list which species your response refers to:

- In our response, we have considered evidence and information pertaining to terrestrial and aquatic farmed species. This information is drawn from members' input and science policy discussions hosted by our Animal Science Group, including most recently at our Animal Science Meeting 2023⁶.
- A general aspect raised during these discussions, which focused more specifically on novel genetic approaches, is the need to develop **species-specific frameworks** to understand the potential benefits and impacts of breeding methods. We heard evidence from companies such as Benchmark

⁶ A copy of the ASM 2023 meeting report can be found on page 29

Genetics, which have several in-house breeding programmes and collaborate globally on 30 breeding programmes in 20 aquatic species⁷. These species have diverse biological features and so a single set of requirements will not suffice. As a consequence, **guidance on the assessment of precision breeding and breeding technologies has to be species-specific.**

- Similarly, in terrestrial farmed species, different breeding sectors will adopt new genetic technologies at different speeds or depth of market penetration. This is partly due to the **market structure and current farming practices.** In the pig, poultry and also the aquaculture sector, uptake of genetic technologies will likely occur more quickly (partly due to a smaller number of research-intensive breeding actors) than for cattle and even more so for sheep, which might take a longer time because of current agricultural practices. However, there is ongoing research into editing cattle genes (see table in appendix 1 on page 26) and high-profile papers were published early on⁸. A research team from Minnesota has an agreement with the company Recombinetics⁹, which through a subsidiary Acceligen¹⁰ is bringing to market the **polled and the slick heat tolerant cattle breeds.** So, there are some promising projects in cattle. Equally there are interesting ideas for genome editing in sheep and goat¹¹, but it will be interesting to see whether any of them is taken to market. We recently hosted a talk that looked at generating new genomic resources to investigate sustainability traits in sheep, which may lead to future genome editing projects across a variety of UK sheep breeds¹².
- We have not covered in great depth the possibility of applying genetic technologies to the **breeding of companion animals**, but we recognise that this may become a likely scenario, given the extensive market that currently exists for pets. Equally, the definition of companion animal can be blurry and a rabbit for example can be both a farmed and a companion animal. We recognise also that UK public opinion, as determined in public dialogue-led studies, is against the use of genome editing for purely aesthetic reasons to generate profit without any additional benefit to an animal's welfare or to society¹³. People may be more prone to accept the use of genetic technologies in companion animals if it addresses a defect that causes ill health or compromises animal welfare, e.g. in some boxer dog breeds cartilage weakness is genetically inherited and this could be targeted with genome editing. Two highly relevant examples of current research are known to us: the use of prime editors to correct a genetic defect in purebred dogs¹⁴ and the creation of "hypoallergenic cats" via genome editing of the feline gene, Fel d 1

⁷ Benchmark Genetics is a world-leader in breeding and genetics for aquaculture. <https://www.bmkgenetics.com/about/benchmark-genetics/innovation>

⁸ Carlson, D.F., Lancto, C.A., Zang, B., Kim, E.S., Walton, M., Oldeschulte, D., Seabury, C., Sonstegard, T.S. and Fahrenkrug, S.C., 2016. Production of hornless dairy cattle from genome-edited cell lines. *Nature biotechnology*, 34(5), pp.479-481.

⁹ <https://recombinetics.com/>

¹⁰ <https://www.acceligen.com/>

¹¹ Kalds, P., Zhou, S., Cai, B., Petersen, B., Sonstegard, T. and Wang, X., 2019. Sheep and goat genome engineering: from random transgenesis to the CRISPR era. *Frontiers in Genetics*, 10, p.465062.

¹² Matika, O., Bishop, S.C., Pong-Wong, R., Riggio, V. and Headon, D.J., 2013. Genetic factors controlling wool shedding in a composite E asycare sheep flock. *Animal genetics*, 44(6), pp.742-749.

¹³ See pages 48-49 Royal Society Public Dialogue on Genetic Technologies. Available at <https://royalsociety.org/news-resources/publications/2018/genetic-technologies-public-dialogue/>

¹⁴ Kim, D.E., Lee, J.H., Ji, K.B., Lee, E.J., Li, C., Oh, H.J., Park, K.S., Lee, S.H., Koo, O. and Kim, M.K., 2022. Prime editor-mediated correction of a pathogenic mutation in purebred dogs. *Scientific reports*, 12(1), p.12905.

chain 2 (CH2), whose protein is a major allergen that causes severe allergic reactions in humans, including rhinitis, conjunctivitis, and life-threatening asthma¹⁵. We would also like to mention **breeding technologies in horses**¹⁶, which, while in limited use in racehorses, are being adopted more widely in polo ponies or competition horses, for example. There is research into genome editing approaches in horses too^{17,18}. Future applications could be welfare-relevant given that several breeds of domestic horse were described as being predisposed to one or more inherited disorders, some of which may have underlying genetic causes¹⁹.

2. What is your interest in agricultural and/or aquicultural breeding practices and/or breeding technologies?

- Our interest lies mainly in **scientific developments** that support better animal health and welfare, sustainable food systems and environmental protection, some of which is carried out by our members at UK research institutions. We acknowledge the pressing need for the **right policy and regulatory environments** that will enable novel breeding methods to be assessed and authorised. This would involve the application of “a proportionate, science-based regulatory system (that) would assess new products by their characteristics, considering the particular traits of a product”²⁰.
- The RSB stated that “in order to protect ecosystems and reduce biodiversity loss, agriculture must do more with less, while adapting to rising global demand and changing climates. No single development can address these complex challenges, and we will need to use all the tools available to deliver the world we need for human survival in acceptable quality conditions”²¹.
- “All forms of breeding have the same goal: to manipulate the genome of an organism to produce a variant with new, desired characteristics”²².

3. What do you consider are the opportunities associated with current selective breeding practices and breeding technologies?

- In a workshop with fellows of the RSB in 2019, we identified **opportunities for novel breeding methods**, including genome editing techniques, to improve on current practices and deliver outcomes such as:
 - making plant and animal breeding more accurate, efficient and better adapted to local needs of human populations and environments

¹⁵ Lee, S.R., Lee, K.L., Song, S.H., Joo, M.D., Lee, S.H., Kang, J.S., Kang, S.M., Idrees, M., Kim, J.W. and Kong, I.K., 2024. Generation of Fel d 1 chain 2 genome-edited cats by CRISPR-Cas9 system. *Scientific Reports*, 14(1), p.4987.

¹⁶ Campbell, M.L.H. and Sandøe, P., 2015. Welfare in horse breeding. *Veterinary Record*, 176(17), pp.436-440.

¹⁷ Pinzon-Arteaga, C., Snyder, M.D., Lazzarotto, C.R., Moreno, N.F., Juras, R., Raudsepp, T., Golding, M.C., Varner, D.D. and Long, C.R., 2020. Efficient correction of a deleterious point mutation in primary horse fibroblasts with CRISPR-Cas9. *Scientific Reports*, 10(1), p.7411.

¹⁸ Moro, L.N., Viale, D.L., Bastón, J.I., Arnold, V., Suvá, M., Wiedenmann, E., Olguín, M., Miriuka, S. and Vichera, G., 2020. Generation of myostatin edited horse embryos using CRISPR/Cas9 technology and somatic cell nuclear transfer. *Scientific reports*, 10(1), p.15587.

¹⁹ Bettley, C.D., Cardwell, J.M., Collins, L.M. and Asher, L., 2012. A review of scientific literature on inherited disorders in domestic horse breeds. *Animal welfare*, 21(1), pp.59-64.

²⁰ Royal Society of Biology (2021). [Response to the DEFRA consultation on the regulation of genetic technologies.](#)

²¹ See ref 20

²² See ref 20

- improving animal health and welfare
- new treatments for human disorders (including new approaches to xenotransplantation^{23,24})
- improving food security and tackling malnutrition
- improving food safety
- improving sustainability (e.g. through more efficient use of land and other resources)
- protecting and preserving biodiversity and ecosystems.
- The necessity to develop and roll-out novel breeding methods is partly driven by the need to **adapt to changing climates and emerging biosecurity risks**²⁵. Equally, the necessity to enhance **sustainability and efficiency in food systems** drives research and development into new breeding methods, including genome editing²⁶, and their potential application to non-intensive agricultural practices²⁷.
- The pace of change in current agricultural practices needed to meet emerging threats is key and “novel genetic technologies have the potential to **accelerate the pace of genetic improvement of livestock species**, particularly through the rational design of novel alleles, and the introgression and promotion of favourable alleles in more efficient ways than through conventional breeding methods”²⁸. “Genome editing could improve rapidity and efficiency in current breeding programmes by avoiding generations of selection within breed, or the need for backcrossing to regain genetic merit after introgression of genes derived from inferior breeds²⁹. If this resulted in an overall reduction of animals involved in breeding programmes, and more refined ways to obtain equal level of genetic gain than current methods, the decision to refuse to use the technology would be ethically questionable”³⁰.
- **Technological interventions should be locally adapted** to the needs and contexts of human and animal populations and their environments. One example is in Africa, which is home to 50% of the world’s smallholder farmers and is particularly vulnerable to emerging climate and biosecurity threats³¹. There are ongoing internationally-led programmes (e.g. under the auspices of the FAO³²), which involve small and medium enterprises (SMEs) and on-the-ground initiatives. Rationalising regulations to provide stable and predictable regulatory pathways for products of modern biotechnologies will support the

²³ Fischer K and Schnieke A (2023), How genome editing changed the world of large animal research. *Front. Genome Ed.* 5:1272687. doi: 10.3389/fgeed.2023.1272687

²⁴ <https://hms.harvard.edu/news/first-genetically-edited-pig-kidney-transplanted-human>

²⁵ FAO. 2015. Climate change and food security: risks and responses. Rome. Available at: <https://www.fao.org/3/i5188e/i5188E.pdf>

²⁶ FAO. 2022. Gene editing and agrifood systems. Rome. <https://doi.org/10.4060/cc3579en>

²⁷ Fernandes, P. M. B., Favarrato, L., Fernandes, A. A. R., Vicien, C., Capalbo, D. M. F., & Zerbini, F. M. (2022). To become more sustainable organic agriculture needs genome editing technology. *Frontiers in bioengineering and biotechnology*, 10, 912793. <https://doi.org/10.3389/fbioe.2022.912793>

²⁸ Royal Society of Biology, 2019. *Royal Society of Biology response to the Nuffield Council on Bioethics call for evidence on ‘Genome Editing and Farmed Animals’*.

²⁹ Eriksson, E., et al. (2018). Breeding and ethical perspectives on genetically modified and genome edited cattle. *Journal of Dairy Science*, Volume 101, Issue 1, January 2018, Pages 1-17. <https://doi.org/10.3168/jds.2017-12962>. See page 6

³⁰ See ref 29

³¹ Advancing biotechnology to solve Africa’s food challenges. <https://www.nature.com/articles/d44148-022-00106-8>

³² FAO. 2019. The status of application, capacities and the enabling environment for agricultural biotechnologies in the Asia-Pacific region. Regional background study. Bangkok. 185 pp. Licence: CC BY-NC-SA 3.0 IGO.

adoption of novel breeding methods in developing, poorly-resourced countries³³.

4. What do you consider are the risks associated with current selective breeding practices and breeding technologies?

- The Nuffield Council on Bioethics's report on 'Genome Editing in Farmed Animals' collated some examples of welfare impacts of **historical breeding practices** in domestic species: for example, the link between 'double-muscling' selection and greater risk of obstructed labour in beef cattle; the muscular and skeletal disorders affecting bigger and faster-growing broiler chickens; or the enhanced occurrence of osteoporosis and bone fractures in laying hens³⁴.
- At an ASG meeting discussion, one of our fellows presented a talk on current issues in dairy cow research and welfare, which included an examination of the links between **genetic selection for milk productivity and health issues in lactating cows**. Over the years, the use of genetic indices for selection and the widespread adoption of artificial insemination using semen from selected sires was a successful strategy to increase milk production and fat content (milk yield increased from 3500 litres per lactation to 15000-16000 litres). However, a concurrent effect of selecting for this trait has been that the fertility and immune systems of cows were compromised. Currently, much more balanced indices have been developed that take into account lifespan, profitable lifetime index, and somatic cells counts all of which help to select for animals that have better health and more resilient phenotypes. Along this line, the British Veterinary Association position on welfare of dairy cows includes the need to give greater importance to genetic selection of non-production traits when breeding dairy cows in order to improve animal health and welfare.
- The existence of **antagonisms between traits** is a point we return to in the answer to the following question on balanced breeding strategies. For example, on the one hand there are traits for production and quality. On the other hand, there are fitness or behavioural traits that are significant from an animal welfare perspective. All these factors must be taken into account when developing breeding strategies or applying biotechnological approaches to achieve desired breeding goals, which would need to be balanced (see answer to question 5). In the case of pig breeding, for example, there is an unfavourable genetic correlation between litter size and piglet mortality: an unbalanced focus on litter size alone, as a key production trait, would lead to increased mortality in piglets. Efforts by breeding companies in the past decades have partly corrected for this adverse correlation and so balanced breeding has led to increased pig litter size while simultaneously increasing piglet survival rates³⁵. Similarly poultry breeders have developed a balanced breeding approach to improve the health and welfare of birds, while selecting for desired production characteristics³⁶.

³³ Adenle AA, Morris EJ, Murphy DJ, Phillips PWB, Trigo E, Kearns P, Li YH, Quemada H, Falck-Zepeda J, Komen J (2018) Rationalizing governance of genetically modified products in developing countries, *Nature Biotechnology* 36, 137-139 <https://www.nature.com/articles/nbt.4069.epdf>

³⁴ Nuffield Council on Bioethics (2021). Genome editing and farmed animal breeding: social and ethical issues. See pages 34-36. <https://www.nuffieldbioethics.org/publications/genome-editing-and-farmed-animals>

³⁵ Knap, P.W., Knol, E.F., Sørensen, A.C., Huisman, A.E., Van Der Spek, D., Zak, L.J., Granados Chapatte, A. and Lewis, C.R., 2023. Genetic and phenotypic time trends of litter size, piglet mortality, and birth weight in pigs. *Frontiers in Animal Science*, 4, p.1218175.

³⁶ Neeteson, A.M., Avendaño, S., Koerhuis, A., Duggan, B., Souza, E., Mason, J., Ralph, J., Rohlf, P., Burnside, T., Kranis, A. and Bailey, R., 2023. Evolutions in Commercial Meat Poultry Breeding. *Animals*, 13(19), p.3150.

- An important environmental concern in the aquaculture sector is **the escape of farmed fish** from pens into open water where they could mate with wild relatives, leading to genetic introgression from farmed animals into wild populations. Scientists and breeders have focused on identifying genetic determinants of sex differentiation and sterility to develop strategies to **minimise risk of interbreeding**. Examples include: germ cell free Atlantic salmon, which were created using genome editing to knock out the Dnd gene^{37,38}, and the Channel catfish, which were engineered for sterility by targeting the luteinizing hormone gene³⁹.

Section 2: Specifically considering breeding selection strategies.

5. Which breeding selection strategies do you consider are beneficial for animal health or animal welfare? Please explain your response.

- In our engagements, scientists and breeders have often referred to **voluntary codes of practices**, which are being adopted by the sector, such as code EFABAR⁴⁰, to support **balanced breeding goals and responsible animal breeding**. Such breeding selection strategies combine several traits, not exclusively linked to productivity, in order to meet the challenges of sustainability in livestock production (see point on antagonism between traits in answer to question 4).
- As an exemplar, Code EFABAR has two parts. The first is **about sustainable breeding** and the second is about the **use of technology**. There are six pillars to the sustainable breeding part: (1) improving animal health and welfare; (2) the reduction of the environmental footprints of animals; (3) improving the quality of the products; (4) more efficient use of resources; (5) maintaining the genetic diversity of populations; and (6) food safety and public health. The second part – on the use of technology – aims to engage with members to deploy technology in a responsible way, to meet the previously mentioned six pillars, but also to consider the impact on the environment and biodiversity. Also, in the same context, to monitor the way that animal breeders use this technology, for the parent breeding stock as well as for the resulting descendants. Code EFABAR also promotes transparency in the way that the technologies are used by companies. We will return to the point of data-sharing and transparency in answer to question 11.
- Discovery and applied research in animal biology remains fundamental to advancing the goals of sustainable animal breeding. It may be difficult to achieve the proposed outcomes without a **better understanding how genetics influence health and welfare of animals in the lab and on the farm**, this includes an understanding of how genes interact with environmental

³⁷ Wargelius, A., Leininger, S., Skaftnesmo, K.O., Kleppe, L., Andersson, E., Taranger, G.L., Schulz, R.W. and Edvardsen, R.B., 2016. Dnd knockout ablates germ cells and demonstrates germ cell independent sex differentiation in Atlantic salmon. *Scientific reports*, 6(1), p.21284.

³⁸ Kleppe, L., Andersson, E., Skaftnesmo, K.O., Edvardsen, R.B., Fjellidal, P.G., Norberg, B., Bogerd, J., Schulz, R.W. and Wargelius, A., 2017. Sex steroid production associated with puberty is absent in germ cell-free salmon. *Scientific reports*, 7(1), p.12584.

³⁹ Qin, Z., Li, Y., Su, B., Cheng, Q., Ye, Z., Perera, D.A., Fobes, M., Shang, M. and Dunham, R.A., 2016. Editing of the luteinizing hormone gene to sterilize channel catfish, *Ictalurus punctatus*, using a modified zinc finger nuclease technology with electroporation. *Marine biotechnology*, 18, pp.255-263.

⁴⁰ <http://www.responsiblebreeding.eu/about-code-efabar.html>

factors^{41, 42}. Additional scientific evidence about the health/welfare of animals bred with the use of novel technologies in real-life conditions on farms is an important stepping stone for policymakers to enact new regulations that would allow animals to enter the wider marketplace. An example of the pathway from discovery research to commercial production is detailed in answer to question 9.

- Ongoing research funded through a European Union Horizon 2020 grant, the HoloRuminant project⁴³, is characterising ruminant-associated microbiomes and evaluating their effect on animal production, health and welfare. We predict that the **integration of microbiome knowledge with breeding management strategies** will grow in importance, for example by identifying the management and feeding practices related with beneficial microbiomes and associated with health and key performance indicators.
- The development of **surrogate sire technologies** is worth considering for their potential to support the integration of genome editing into breeding programmes at scale⁴⁴. Surrogate sire technology enables the creation of males that lack their own germline cells, through knock-out of *NANOS2* in mammals that are then recipients of spermatogonial stem cells from other donor males⁴⁵. Once the genetically sterile surrogate receives the precursors of sperm cells from the 'elite' genetic donors, they can disseminate the traits of interest to a large number of offspring by natural breeding. In the chicken knockout of the gene *DDX4* results in both males and females that are sterile, generating surrogate hens and cockerels that become fertile hosts by transfer of germline stem cells, from either different breeds or gene edited cells. Laboratory testing of these technologies was carried out in pigs, goats, mice and chicken (see ref 44).
- While such **integration of genome editing into breeding programmes** provides an important opportunity for trait improvement, its application will require **innovative solutions**, as discussed in a recent review⁴⁶. The review paper discussed modelling research into how a single 'elite' male donor animal could produce huge numbers of progeny in livestock breeding programmes⁴⁷. The use of surrogate sire technology would significantly increase the genetic merit of commercial sires by as much as 6.5 to 9.2 years'-worth of genetic gain in comparison to conventional commercial breeding, according to the simulations and depending on the species under consideration. However, an emerging risk that will need to be mitigated is the **potential for high levels of inbreeding**, if only one or a handful of 'elite' donor animals are used to generate the production animals in the breeding programme.

⁴¹ Tiezzi, F. and Maltecca, C., 2022. Genotype by environment interactions in livestock farming. In *Animal Breeding and Genetics* (pp. 77-97). New York, NY: Springer US.

⁴² Rauw, W.M. and Gomez-Raya, L., 2015. Genotype by environment interaction and breeding for robustness in livestock. *Frontiers in genetics*, 6, p.157050.

⁴³ <https://holoruminant.eu/project/objectives/>

⁴⁴ Clark, E.L., 2022. Breeding in an era of genome editing. *Animal Breeding and Genetics*, p.369.

⁴⁵ Giassetti, M.I., Ciccarelli, M. and Oatley, J.M., 2019. Spermatogonial stem cell transplantation: insights and outlook for domestic animals. *Annual review of animal biosciences*, 7, pp.385-401.

⁴⁶ See ref 44

⁴⁷ Gottardo, P., Gorjanc, G., Battagin, M., Gaynor, R.C., Jenko, J., Ros-Freixedes, R., Bruce A. Whitelaw, C., Mileham, A.J., Herring, W.O. and Hickey, J.M., 2019. A strategy to exploit surrogate sire technology in livestock breeding programs. *G3: Genes, genomes, genetics*, 9(1), pp.203-215.

- Similarly in aquaculture, technical implementation of genome editing into animal breeding at scale is a critical step. Current editing methods used in research settings, based on microinjection of F0 generation embryos, are laborious, inefficient, and result in highly mosaic animals. The need for scalability without embryo mosaicism led researchers to **develop surrogate broodstock technologies**⁴⁸. When combined with appropriate implementation plans for genetic evaluation, selection and performance testing in the breeding nucleus, these technologies can accelerate genetic gain, and improve dissemination of elite germplasm.
- We will discuss below (Section 3.9) the development of pigs resistant to the porcine reproductive and respiratory syndrome (PRRS) virus by means of targeted genome editing and their regulatory approval for commercialisation currently underway in the US⁴⁹. In this example of the application of novel breeding technologies, there are additional direct and indirect benefits beyond animal health and welfare, including for the livelihood of farmers affected by disease outbreaks. Disease resistance would improve productivity, reduce waste and improve the environmental footprint of pig production. An important societal gain from PRRS viral resistance is the **reduced use of antimicrobials** in pig farming. When the animals' immune cells are infected, the animals become immunosuppressed and more vulnerable to secondary bacterial infections, which require antibiotics.

6. Are you aware of current selection strategies that may be harmful for animal health or animal welfare? Please explain your response.

- Please see answer to question 4 and the need to develop balanced breeding programmes that select for both productivity-related and fitness/animal welfare-related traits at the same time.

7. If you or your organisation uses or espouses specific selection strategies, how do you ensure that animal health harms or animal welfare harms are minimised for all individuals?

- At the last Animal Science Meeting 2023, several discussions revolved around the **animal welfare assessment framework**, which will be part of secondary legislation and regulatory guidance under the GT(PB)A 2023.
- Important questions to consider are: how will scientists, breeders and farmers assess the impact of specific genomic changes/traits on an animal's ability to live with a good standard of life; how might the effect on the animal depend on the environment in which it might be kept; and what are the key aspects of welfare that may be compromised or enhanced?
- The Five Domains model⁵⁰, which includes both **physical and mental states** and includes both **positive and negative welfare**, is an accepted basis for the assessment of an animal's welfare and quality of life, but there is no single

⁴⁸ Jin, Y.H., Robledo, D., Hickey, J.M., McGrew, M.J. and Houston, R.D., 2021. Surrogate broodstock to enhance biotechnology research and applications in aquaculture. *Biotechnology Advances*, 49, p.107756.

⁴⁹ <https://www.ed.ac.uk/roslin/facilities-resources/larif/case-studies/industry-partners>

⁵⁰ Mellor, D.J., Beausoleil, N.J., Littlewood, K.E., McLean, A.N., McGreevy, P.D., Jones, B. and Wilkins, C., 2020. The 2020 five domains model: Including human–animal interactions in assessments of animal welfare. *Animals*, 10(10), p.1870.

agreed methodology. Whatever method is used, it needs to be tailored for the species and the type of animal use, and to be easy to use.

- Ideally, assessments should be made **at the animal level**, rather than simply consider provision of resources. Basic animal welfare assessment does not always cover all of the Five Domains; because behaviour and mental state are not routinely assessed, the animal's physical condition tends to dominate the assessment. Therefore, particular focus should be given to define **appropriate behavioural indicators**. As well as including the effects of gene editing on health, growth and behaviour, it is also necessary to evaluate the impact of the environment on the animal. This process should continue across the animal's lifetime in order to capture any potential delayed effects of gene editing. Monitoring and assuring the welfare of animals on farms will be critically dependant on farmers' skills and resources as mentioned below. This is a critical factor to consider in any realistic assessment of the impact of breeding technologies on animal welfare.
- During the **early experimental phase**, the Animals (Scientific Procedures) Act 1986 and associated ethical and regulatory requirements determine where genome editing work on animals can be licensed following a harm-benefit analysis. The application of the 3Rs is a fundamental guiding principle to minimise suffering while attempting to accrue benefits and knowledge from the research. During this phase, a large number of indices for potential animal welfare risk factors could be evaluated under research testing conditions. Under controlled conditions, one can record sample size of test and control groups, the number of generations included in the analysis, and relevant information on age, sex and other factors likely to affect the experimental outcome.
- Animals used for a research study are likely to be maintained in relatively small numbers, and in high quality facilities with optimal nutritional, health and environmental management whereas, on **commercial farms**, animals will probably be kept at higher stocking density, with variable nutrition, health and environmental management and potentially exposed to variable impacts of environmental temperature, relative humidity and air quality, for example.
- The challenge is to define the appropriate **post-marketing monitoring of relevant indicators on a farm** and agreeing a reporting system that is manageable for farmers and effective in informing scientists, breeders and any regulatory authority involved. To ensure that the gene edit does not interact with variable environments in a negative way for welfare, monitoring of animals in a range of commercial environments will be essential. At this commercial stage, the number of measured indicators will necessarily be fewer than those of the experimental phase. The right tools to carry out the animal welfare assessment should be available to farmers and we touch on this aspect in answer to question 9.
- At ASM 2023, a roundtable considered the evolution of breeding programmes in the genomic area and suggested that breeding outcomes, traits, welfare outcomes and the context of the species/productions systems are all important factors to consider. However, outcomes depend on the choice of indices: what and how we decide to measure and under what conditions? At ASM 2023, there was a debate about the kind of indicators that should be used in the experimental versus commercial settings. **Welfare assessment/ indicators**

will likely need to be **bespoke** to each trait or species. There should be both hypothesis-driven testing (to be determined on a case-by-case basis) and holistic assessment of basic welfare variables. Regulations of precision bred animals should aim to reach a level playing field and proportionality, with respect to how current breeding processes are regulated. The GT(PB)A 2023 sets the stage for new regulations but data requirements need to be targeted. There should be alignment between the global regulatory/breeding context and the need for data collection under the new Act.

- At a recent meeting of the ASG, an expert on fish welfare, Dr Ruth Clements (VetSustain), presented a talk on how the aquaculture breeding sector is focusing on defining and monitoring **positive welfare outcomes in finfish**. A framework is being developed that looks at the lifetime experience of the animals in a system on the basis that there could be various outcome measures over their lifetime. This should allow comparison between different farms, pens, and systems. The sector is learning more about welfare outcome measures that might feed into this more holistic assessment of the animal's whole life experiences.
- In 2019, the ASG hosted an invited talk with Dr Lynne Sneddon on **pain and welfare assessment in fish**, which included an up-to-date review of the evidence for pain processing in fish. Researchers developed software for analysis of 3D videos, to assess the pain of zebrafish in a laboratory setting, based on their motor patterns while swimming in a tank. It would be interesting to explore if such an animal welfare assessment system devised for use in laboratory conditions could be adapted for use on fish farms in affordable and sustainable ways.
- There are **knowledge gaps on what positive welfare looks like for the various species**, for example in aquaculture. Migratory behaviour of salmon from a fresh to a marine environment and back again occurs in wild animals. This behaviour is prevented in captivity. Researchers do not fully understand enough about the importance of this behaviour in this particular species, and indeed, in many farmed species. They are still learning about some of the high motivation behaviours and how farmers can provide optimal environments and enrichment for each species. Dr Clements has recently been involved in writing a policy position on sustainable finfish production with the BVA, which gives many more details on welfare and wider sustainability⁵¹.
- ASM 2023 participants also suggested that there is need for **better transparency of breeding data** and its accessibility for animal welfare researchers (and other stakeholders). Specifically, this should apply to how breeders share data that are relevant to animal welfare. The Animal Welfare Declaration in the new Act provides an opportunity to improve on the *status quo*.
- There was a conversation about the role of **assurance schemes** linked to the animal welfare assessment and the potential need for **educational/training opportunities for farmers**, who could learn robust monitoring and reporting systems for high level welfare indicators. No clear solutions were presented but a suggestion was to look at the training/qualifications for animal technicians in the context of laboratory science, as an example. Other suggestions were: to

⁵¹ British Veterinary Association (2023). [Policy position on UK sustainable finfish aquaculture – Executive Summary](#).

add specific training into curricula in agriculture, e.g. similar to the requirement to attend a training course for each pesticide, or GE breeding companies could train farmers in welfare and keeping of animals. There is currently a lack of focus and opportunities for upskilling of farmers and people employed to look after the animals, which will represent a practical barrier to any improvement of animal welfare on the ground. In the case of antimicrobial use (AMU) and stewardship, farmers were more likely to have better knowledge of antimicrobials and AMR if they had undertaken a university degree. However, from the available knowledge “it is believed that producers holding higher academic qualifications represent a small proportion of the Scottish dairy sector” so strategies to improve correct antimicrobial use should “focus AMU training and AMR awareness-raising activities towards younger, less experienced farmers as well as those with a lower educational qualification (high school vs. university degree)”⁵².

Section 3: Specifically considering current and future technologies that may be used as part of a breeding programme.

8. Are there any specific technological advances over the last 10 years and/or may be introduced in the next 10 years that you think AWC should include in this review?

- **Livestock genetic improvement** is enabled by the confluence of “objective phenotyping, genomic information, statistical methodologies and advanced reproductive techniques”⁵³. The goal is to be able to use the information in an animal’s genome to predict its phenotype more accurately.
- **Genomic science** has advanced significantly in the last decade, and sequencing technologies and computational methods currently available have become more powerful and affordable. In a talk delivered at the Animal Science Meeting 2023, Dr Emily Clark (Roslin Institute) described how advances in the analysis of genome function are providing tools and knowledge to answer the **genotype-to-phenotype** question⁵⁴.
- The **Functional Annotation of Animal Genomes (FAANG) project**⁵⁵ is an international effort to characterise the functional elements of the genomes of farmed animals. The first stages of FAANG focused on foundational data generation to characterise expressed and regulatory genomic regions, curation and provision of highly annotated farmed animal genomes⁵⁶. These were largely based on high depth approaches at the individual level. The primary challenge facing this community now is harnessing both the resources generated, and the potential of new technologies, to link genotype, phenotype

⁵² Borelli, E., Ellis, K., Pamphilis, N.M., Tomlinson, M. and Hotchkiss, E., 2023. Factors influencing Scottish dairy farmers’ antimicrobial usage, knowledge and attitude towards antimicrobial resistance. *Preventive Veterinary Medicine*, 221, p.106073.

⁵³ Bishop, T.F. and Van Eenennaam, A.L., 2020. Genome editing approaches to augment livestock breeding programs. *Journal of Experimental Biology*, 223(Suppl_1), p.jeb207159.

⁵⁴ See ASM 2023 report on page 29

⁵⁵ <https://www.animalgenome.org/community/FAANG/>

⁵⁶ Archibald, A.L., Bottema, C.D., Brauning, R., Burgess, S.C., Burt, D.W., Casas, E., Cheng, H.H., Clarke, L., Couldrey, C., Dalrymple, B.P. and Elvik, C.G., 2015. Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project: open letter. *Genome Biology*, 16.

and genetic merit in order to translate this research from the laboratory into industrial application in the field⁵⁷.

- To achieve advancements in genotype-to-phenotype predictions, researchers will “need to generate functional genomic information for **large populations of animals**, rather than relying on a small number of deeply annotated individuals. Furthermore, to date, most of the datasets are from tissues consisting of heterogeneous cell populations, hindering the resolution of functional information and limiting our ability to understand the fundamental cellular and subcellular processes underlying phenotypes”⁵⁸.
- The next stage of the FAANG project (**FANG to Fork strategy**) could lead to substantial innovation in the farming sector; its research priorities for the next decade are represented in a diagram in appendix 2 on page 27 and summarised here:
 - development of tools and resources for new breeding technologies such as genome editing. These developments require **high-throughput in vitro systems** to model genome-to-phenotype links and hypothesis-testing through perturbation experiments using genome editing.
 - **pan genomes and comparative genomics** to fully discover, preserve and utilize genome diversity. An interesting application is the collection of new genomic resources for indigenous and sustainable populations of livestock species in the UK (such as sheep for example⁵⁹).
 - large cohorts of animals with data to characterise genotype-to-expression in many **tissues and cell types**;
 - **large phenotype collections** gathering data from many animals raised in well-defined environments to study variations in traits that have health and welfare impacts;
 - **biobanking** as a resource for research and conservation using in vitro biorepositories. iPSCs and organoids that reflect species, breed and population diversity⁶⁰.
 - development of **single-cell atlases** to build tissue, species and life stage specific functional maps at cellular resolution, particularly for traits linked to specific cell types (tissue-specific and cell-type specific omics)⁶¹.
- The set of experimental and computational technologies that will enable accurate **functional annotation of animal genomes** has expanded in the past decade and will still grow⁶².

⁵⁷ Clark, E.L., Archibald, A.L., Daetwyler, H.D., Groenen, M.A., Harrison, P.W., Houston, R.D., Kühn, C., Lien, S., Macqueen, D.J., Reecy, J.M. and Robledo, D., 2020. From FAANG to fork: application of highly annotated genomes to improve farmed animal production. *Genome Biology*, 21, pp.1-9.

⁵⁸ See ref 57

⁵⁹ Ensembl in a new era - deep genome annotation of domesticated animal species and breeds. BBSRC Porfolio analyser: <https://gow.bbsrc.ukri.org/grants/AwardDetails.aspx?FundingReference=BB/W018772/1>

⁶⁰ We need to preserve biobanks or pools of diverse animals before they disappear from farms because that diversity will be precious to respond to future challenges – almost like keeping a reference library for livestock species.

⁶¹ Examples of recent funded grant proposals can be found here: <https://www.animalgenome.org/community/FAANG/proj.php>

⁶² See the evolution of the **Encyclopedia of DNA Elements (ENCODE) project** from 2003 to now. The “recognition of the need for new approaches, methods and technologies to achieve the goals of ENCODE, NHGRI has also funded four rounds of technology development initiatives since 2003. A number of these efforts have been incorporated into subsequent phases of ENCODE data production and analysis. In all its phases, data sharing and information dissemination have been high priorities – success in these efforts is reflected by the greater than 2,000 publications from non-ENCODE researchers who have to date used ENCODE data for their studies”. <https://www.genome.gov/about-nhgri/Director/genomics-landscape/August-6-2020-NHGRI-completes-phase-3-of-encode-project>

- Another area where advances will elucidate the genome-to-phenotype relationships, particularly in response to environmental signals, is the study of the **epigenome** in farm animals⁶³ and developing better understanding of factors controlling cell type specific gene expression. As described in a recent review, “the bulk of available data mainly characterized the epigenetic markers in tissues/organs or in relation to traits and detection of epigenetic regulatory mechanisms underlying livestock phenotype diversity. However, available data are inadequate to support gainful exploitation of epigenetic processes for improved animal health and productivity management”⁶⁴ at this stage.
- Understanding of the **microbiome** and its impact on host physiology, health and welfare will also drive innovation in the farming sector. The ongoing Holoruminant research project “use a holistic multi-omics approach to characterize the establishment and dynamics of microbiomes. [...] (it) will determine the connectivity between microbiomes from different body sites, their heritability and their influence on the host’s key performance indices (KPIs) of efficiency of production, growth, resistance to disease, methane emissions, carbon footprint and phenotypic resilience to changing environmental conditions”⁶⁵.
- There are also ongoing research efforts to extend the available databases from a single reference genome to **population level genomes** (see for example: AcquaFAANG⁶⁶, BovReg⁶⁷, GeneSwitch⁶⁸).
- The use of genome editing can be combined with our increasing knowledge of livestock genomics in a range of options and applications⁶⁹, such as:
 - the detection and utilisation of causative genetic variants that affect important traits;
 - the “introgression-by-editing” of favourable naturally occurring alleles into closed breeding populations;
 - or through creation of de novo alleles with desirable effects.
- Alongside advances in genomic science, new **technologies for monitoring phenotypic variation** are needed, with many currently in development. These technologies include the use of sensors, data acquisition and analysis software to collect “routine laboratory data at many different times in an individual’s life in several livestock species. [...] The research community is embracing the challenges in computation and software development for precision livestock

⁶³ Ibeagha-Awemu, E.M. and Khatib, H., 2023. Epigenetics of livestock health, production, and breeding. In Handbook of Epigenetics (pp. 569-610). Academic Press.

⁶⁴ Wang, M. and Ibeagha-Awemu, E.M., 2021. Impacts of epigenetic processes on the health and productivity of livestock. *Frontiers in Genetics*, 11, p.613636.

⁶⁵ <https://holoruminant.eu/project/objectives/> In more detail, some of the work streams will try to: (i) Understand the dynamics of microbiomes: through the transfer of microbes from the dam (vertical) and farm environment (horizontal) and within the microbiomes at different body sites of the same individual. (ii) Characterize microbiome roles during challenging life periods such as perinatal, weaning, and following exposure to pathogens. (iii) Integrate microbial markers and host genetics into statistical models for identifying phenotypic variance.

⁶⁶ <https://www.aqua-faang.eu/>

⁶⁷ <https://bovreg.eu/>

⁶⁸ <https://www.gene-switch.eu/eurofaang.html>

⁶⁹ Royal Society of Biology, 2019. Royal Society of Biology response to the Nuffield Council on Bioethics call for evidence on ‘Genome Editing and Farmed Animals’. See paragraph 1.2 on page 4.

phenotyping tools. However, adoption in the field by producers industry, and veterinary practitioners will mark the full realization of its potential”⁷⁰.

- New digital and **artificial intelligence technologies** also offer the potential to monitor phenotypic variation in traits that affect animal health or welfare, over multiple generations and at a farm level. The application of these technologies to an animal welfare assessment of choice will help to build a realistic picture of how breeds fare in real life conditions. There will be challenges for the collection of high-quality phenotyping data on-farm, in great part due to how farmers interact with the tools, hence the importance of co-design and involvement of end-users in technological development from the early stages.
- Genomic management Tools to Optimize Resilience and Efficiency (GenTORE) is developing innovative genome-enabled selection and management tools to empower farmers to optimize cattle resilience and efficiency (R&E) in different and changing environments⁷¹ - an example of **precision livestock farming** (PLF) technology-assisted approaches. In a recent publication⁷², the GenTORE team describes how “using available on-farm technology allows large-scale phenotyping of resilience and efficiency that can be applied to evidence-based management, breeding and culling decisions. Veterinarians and other farm advisors are engaged with farm business drivers that are influenced by consumer and societal demands including the environment, human health concerns regarding antimicrobial resistance and animal welfare”. They also describe the existing conflicts between these factors in real life situations and how new tools can combine “multiple streams of sensor data [...] to inform herd-level strategy in a time-efficient, automated and objective system to support advisor input to herd health”.
- Advancement in genomics, gene editing and associated reproductive technologies may **allow breeders to move faster**, and for a greater number of traits, acting on those aspects of the breeders’ equation that are not normally accessible via traditional breeding (e.g. through improvement of the accuracy with which we can predict the breeding values for a given trait and the introduction of genetic variation not seen as accessible in a given population).
- Advances in effective **multiplexing** of genome editing, where **polygenic traits** are altered in a single step, will be “required to enable multiple edits in elite breeding animals within a breeding nucleus to target multiple traits or multiple causative alleles for the same trait”⁷³. These technologies should become available in the future as molecular methods become more refined with the benefit that “introducing edits into multiple elite animals, into a breeding programme, will [...] avoid genetic bottlenecks and editing of different breeds

⁷⁰ Koltjes, J.E., Cole, J.B., Clemmens, R., Dilger, R.N., Kramer, L.M., Lunney, J.K., McCue, M.E., McKay, S.D., Mateescu, R.G., Murdoch, B.M. and Reuter, R., 2019. A vision for development and utilization of high-throughput phenotyping and big data analytics in livestock. *Frontiers in genetics*, 10, p.1197.

⁷¹ <https://www.gentore.eu/>

⁷² Statham, J.M.E., Burton, K.L., Adriaens, I., Lora, I., Cozzi, G., de Haas, Y., Kamphuis, C., Vedder, L., Loke, B. and Friggens, N.C., 2022, December. Developing precision livestock farming in practice: using sensor time series data for breeding decision support systems. In *Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) Technical and species orientated innovations in animal breeding, and contribution of genetics to solving societal challenges* (pp. 659-662). Wageningen Academic Publishers.

⁷³ Clark, E.L., 2022. Breeding in an era of genome editing. *Animal Breeding and Genetics*, p.369.

and lines will be essential to maintain genetic diversity, and enable structured cross-breeding”⁷⁴.

- As regulations are developed under the GT(PB)A 2023, similarly to the [ACRE technical guidance for making higher qualifying plants](#) (HQP), which describes how genome editing (GE) applications in plants can be compared to traditional breeding and therefore qualify for an application under the new Act. It will be important that any advice gathered through this call for evidence, which pertains to the **type of traits/mutations currently bred** in livestock species, is discussed with ACRE and relevant teams at DEFRA to make sure that clear guidance is delivered to and co-designed with the sector. This is essential to ensure that clarity is achieved about the kind of mutational projects that ‘could have arisen spontaneously or in traditional breeding’.
- On the question of techniques, we would like to stress the importance of **quality control standards** in the research and development phase of precision bred animals (see also our previous communication to the Nuffield Council on Bioethics⁷⁵). At a discussion of the RSB Animal Science Group, we considered the lessons being learned in the field of laboratory mice genetics, where there is debate about basic standards for quality control in genome edited mice particularly in respect of template integration, off targets effects and mosaicism in founder lines. Working with livestock animals versus laboratory inbred strains has its specificities but referring to parental genomes as a reference for detecting unintended genomic effects from the editing will be important. The experts agreed on the need to devise the correct pipeline for validations through deep quality controls of founder animals.

9. Are there any current or future technologies that could be utilised as part of a breeding programme that you consider would be beneficial for animal health and/or animal welfare? Please explain your response.

- The development of **farm animal organoids** could support the 3Rs (Replacement, Reduction and Refinement) in the context of research into farm animal breeding, health and welfare, but also in the context of translational medicine and One Health. A recent review looked at the development of farm and companion animal organoids and their potential in public health, food security, and comparative medicine: “farm animal organoids could play an important role in investigations of the pathophysiology of zoonotic and reproductive diseases by contributing to public health and improving agricultural production”⁷⁶. Ongoing research is aimed at creating “an organoid biobank with different species of farm animals and making it available to the entire scientific community that studies infectious diseases of zoonotic origin in humans and other animals”⁷⁷.

⁷⁴ Clark, E.L., 2022. Breeding in an era of genome editing. *Animal Breeding and Genetics*, p.369.

⁷⁵ Royal Society of Biology, 2019. [Royal Society of Biology response to the Nuffield Council on Bioethics call for evidence on 'Genome Editing and Farmed Animals'](#). See paragraph 1.5 on page 6.

⁷⁶ Kawasaki, M., Goyama, T., Tachibana, Y., Nagao, I. and Ambrosini, Y.M., 2022. Farm and companion animal organoid models in translational research: a powerful tool to bridge the gap between mice and humans. *Frontiers in Medical Technology*, 4, p.895379.

⁷⁷ <https://www.irta.cat/en/spains-first-farm-animal-organoid-biobank-will-be-set-up-at-the-irta-to-allow-research-into-infectious-diseases-without-the-use-of-live-animals/>

- The table in appendix 1 on page 26 (from ref 44) provides examples of **genes targeted to improve different categories of traits using genome editing tools**. Traits like resistance to pathogens and polledness have direct positive impacts on animal health and welfare. **Resistance to pathogens** is a major opportunity for application of gene editing, as selective breeding approaches to identify resistance to individual pathogens particularly in terrestrial farmed animals is a major challenge. Robust disease resistance would be a major animal welfare benefit. Similarly in the aquaculture sector, infectious disease presents a persistent threat with negative impacts on animal welfare, and the economy, and can lead to environmental concerns. In aquaculture it is more difficult to handle individual animals and vaccination, and biosecurity or other control measures are often not feasible, so genetic approaches could prove particularly beneficial.
- An interesting case study for the application of genome editing to introduce genetic resistance in livestock species is the generation of **Porcine reproductive and respiratory syndrome (PRRS) virus resistant pigs** developed at the Roslin Institute^{78,79}. PRRS is a viral disease of pigs that is characterised by miscarriages, death of newborn animals and respiratory deficiencies in older animals. It leads to a huge economic cost for the pig industry – in the EU alone the economic loss amounts to €1.5 billion annually. There are vaccines available but they are not effective for all variants of the virus. The inactivated virus in the vaccine may revert to virulent forms causing outbreaks in vaccinated animals. PRRS virus infects lymphocytes and macrophages by latching on a protein on the cell surface, the CD163 receptor. This protein has nine globular domains. Domain 5 is the binding site for PRRSV, which is encoded by exon 7. CD163 has many important biological functions so a complete gene knock-out (KO) would probably not be a good strategy for production animals (see discussion of potential concerns in answer to question 10). The Roslin team therefore designed a targeted approach that would remove only domain 5 of the cellular receptor via genome editing and tested this strategy *in vivo*. The genome edited pigs were challenged with a highly virulent strain of the PRRS virus and in contrast to the wild type control group, they showed no signs of infection, no viremia or antibody response. Further analysis confirmed that the animals were fully resistant to infection by the virus.
- The University of Edinburgh has filed a patent to protect the IP exemplified by the demonstrated proof-of-principle and the University Technology Transfer team has negotiated with a leading pig breeding company a licence to the technology. The company has established a subsidiary to validate phenotype in a large cohort of animals and is working with the FDA (in the US) regarding the regulatory position and predicts an overall 6-year lead-in to market.
- The **breeding company Genus PIC** has generated founder animals with the same mutation and bred them to produce the next three generations of pigs to establish multiple lines of pigs homozygous for the edited allele. They confirmed that the CD163 gene with removed exon 7 was stable during multiple

⁷⁸ Burkard, C., Lillico, S.G., Reid, E., Jackson, B., Mileham, A.J., Ait-Ali, T., Whitelaw, C.B.A. and Archibald, A.L., 2017. Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLoS pathogens*, 13(2), p.e1006206.

⁷⁹ Burkard, C., Opriessnig, T., Mileham, A.J., Stadejek, T., Ait-Ali, T., Lillico, S.G., Whitelaw, C.B.A. and Archibald, A.L., 2018. Pigs lacking the scavenger receptor cysteine-rich domain 5 of CD163 are resistant to porcine reproductive and respiratory syndrome virus 1 infection. *Journal of virology*, 92(16), pp.10-1128.

breeding cycles. A recently published paper⁸⁰ by Genus PIC scientists evaluated the PRRS virus resistant pigs relative to non-edited animals from birth to maturity, including any potential changes in meat composition and resistance to PRRSV.

- We would encourage the AWC members to look into the details of the paper, including the animal health and welfare variables that were assessed. The RSB Animal Science Group would welcome the opportunity to organise a follow-up discussion with experts, should the committee appreciate discussing the implications of this work for the development of UK regulations for the GT(PB)A animal welfare assessment framework.
- At the Animal Science Meeting 2023 (see report on page 29), experts and participants discussed **genome editing approaches for zoonotic resistance**. It was recognised that biosecurity conditions, albeit within a common regulatory framework, will vary in effectiveness from farm to farm. Genome editing could be an efficient and safer way to deal with infectious diseases. The current threat of antimicrobial resistance (AMR) also makes genome editing an attractive solution. Furthermore, some diseases elude vaccine development despite decades of research. Please see answers to question 10 for concerns/potential risks of editing genes for pathogen resistance and to question 11 for additional biosecurity/One Health concerns that may need to be addressed by regulations under the GT(PB)A 2023.
- In answer to question 7 we introduced the necessity of a robust, adaptive and species-specific **animal welfare assessment** in the context of current and future breeding practices. This principle should inform the development and adoption of **practical tools** that can be rolled out on real farms to monitor individual animals' quality of life through their life span. The involvement of end-users (farmers and breeders) in the co-designing of tools is recommended to ensure their future success and utility in practice.
- At the ASM 2023, participants discussed one possible approach for collecting data and monitoring animals based on the **Animal Welfare Assessment Grid (AWAG)**, which encompasses Mellor's five domains of welfare across four parameters: physical, behavioural/psychological, environmental and procedural⁸¹. The AWAG considers the lifetime experience of the animal and the cumulative suffering that can impact quality of life. The tool provides a mean score for factors in each parameter and plots these on a grid to create a minimum convex polygon, the area of which is the cumulative welfare assessment score (CWAS) for that moment in time. The CWAS is then presented plotted against time, and can be tracked across the animal's lifetime

⁸⁰ Nesbitt, C., Galina Pantoja, L., Beaton, B., Chen, C.Y., Culbertson, M., Harms, P., Holl, J., Sosnicki, A., Reddy, S., Rotolo, M. and Rice, E., 2024. Pigs lacking the SRCR5 domain of CD163 protein demonstrate heritable resistance to the PRRS virus and no changes in animal performance from birth to maturity. *Frontiers in Genome Editing*, 6, p.1322012.

⁸¹ Description of the four parameters:

Physical: assesses an animal's clinical health including factors such as body condition, illness and injury.

Behavioural/Psychological: assesses an animal's mental wellbeing and includes factors such as behavioural response to stressors and how often these are encountered. Animals cannot verbally communicate their emotions, therefore behaviour is used as an indicator to explore their psychological health.

Environmental: assesses the animal's environment, whether it is both suitable and complex, options for social opportunities, along with choice and comfort.

Procedural: assesses how the animal responds to clinical and husbandry events, and includes factors such as handling, change in routine, and pain from veterinary or management procedures.

to assess quality of life, allowing the user to quantify welfare and assess if treatment or changes in management systems are required or have been successful in improving welfare. The AWAG allows the user to drill down and identify which factors are positively or negatively affecting welfare and make appropriate focused interventions. This tool has been used for a wide variety of species and various environments, including zoos, farms, companion animal care and research laboratories (see reference list on www.awag.org.uk). The final AWAG is species- and use-specific and consists of defined factors, within the four parameters, each scored incrementally on a scale from 1 (best welfare state) to 10 (worst welfare state). Although welfare is a subjective experience, assessing welfare at group-level leaves individual differences and individual personality unaccounted for. Group-level welfare assessments tend to focus more on what the animal has been provisioned with, harking back to the 'Five Freedoms', rather than assessing animal-based measures such as individual behaviour and physical and psychological health. However, through using the AWAG model, animal-based measures have been included in this group-level welfare assessment and welfare trends over time can be identified and tracked. The results also show that it is possible to identify which factor(s) may be impacting welfare, allowing animal care givers to focus on improving these.

- There is potential **synergy between Precision Livestock Farming tools and Animal Welfare Assessment systems** that the Committee should investigate in order to define the data requirements and right implementation approaches, which DEFRA could consider when drafting new regulations under the GT(PB)A. Tools enabled by phenotyping and AI technologies may become effective in guiding other husbandry and veterinary choices on farms, for example when monitoring the likelihood of disease or efficacy of treatment interventions.

10. Are there any current or future technologies that could be utilised as part of a breeding programme that you consider could be harmful for animal health and/or animal welfare? Could these technologies be adapted to minimise potential animal health and/or welfare harms? Please explain your response.

- In our response to the 2021 DEFRA consultation on future regulation for genetic technologies, we stated that: “in animal breeding, genome editing is a fast evolving technology that can refine and expand current breeding practices, for example by introducing de novo favourable alleles. However, genome editing can still lead to unwanted artefacts that must be carefully checked for with appropriate validation strategies”⁸². However, only some of these unwanted artefacts may lead to any animal health/welfare impacts. In paragraphs 3.16-17 we looked at the risks of genome editing for animal welfare in more detail, with a specific mention of **assisted reproduction techniques** (ARTs). Since our initial response to the Nuffield Council on Bioethics consultation in 2020, several papers have shown improvements in ARTs efficiency and impacts on animal welfare – this is an area of research that should be monitored for progressive refinements, alongside the development of surrogate sire and other

⁸² See ref 20, para 3.17-3.18 on pages 15-16.

reproductive approaches to extend genome editing approaches to large scale animal production.

- An additional aspect of concern that we raised previously is that “animal welfare assessment to be conducted in a GE project can be complicated by our limited **understanding of the full spectrum of what different genes do**. Genes can often have different roles in different tissues and are subject to complex tissue-specific regulatory mechanisms⁸³. Additional research and technological advances will shed light on the underlying biological complexity, therefore a future regulatory system should be agile and proportionate in managing risks for animal welfare, should potential hazards be identified”⁸⁴.
- We would like to discuss here the case of the **PRRS virus resistant pigs** (see answer to question 9) in more detail, in response to concerns brought forward by some of our members. Because genetic resistance in the edited pigs is mediated by deletion of domain 5 of the CD163 receptor present on cells of the monocyte/macrophage lineage, we heard **concerns about potential deficiencies in immunological function** in these animals, particularly those affecting the inflammatory response and wound healing, which in turn could lead to bruising, unwanted impacts on meat quality or cause altered behaviours due to defective wound healing. The complete knock out of CD163 in laboratory mice is linked to changes in muscle regeneration and repair following ischaemic injury⁸⁵ and a significantly lower survival rate in CD163-deficient mice to endotoxin/septic shock, due to insufficient suppression of inflammatory responses⁸⁶. In pigs, the deletion of domain 5 as opposed to the whole gene preserves critical functionalities of the CD163 protein, particularly those associated with the function of the soluble CD163, as a master orchestrator of the inflammatory response. In appendix 3 on page 28 we draw together a few references and considerations from our members relating to CD163 wider physiological roles and in the context of the GE pig project.
- This is just one example of the **complexities involving immunological processes** which may emerge when considering genome editing approaches for disease resistance in livestock. It would be valuable for the Committee to consider a few case studies of how genome editing is applied to disease resistance in farmed animals, focusing on the specific genes and their wider functions in physiology and pathology. Furthermore, in research and development phases GE animals are challenged by single pathogen types under controlled (often high quality husbandry) conditions. In real farm conditions, animals will be exposed to multiple different pathogens and environmental challenges, hence the importance of carefully thought-through regulatory approval pathway from proof-of-concept stage to commercial release, which may include data collection from a variety of farms over a longer period of time post release. Data and regulatory requirements should be

⁸³ Royal Society of Biology, 2019. [Royal Society of Biology response to the Nuffield Council on Bioethics call for evidence on 'Genome Editing and Farmed Animals'](#). See point 1.4

⁸⁴ See ref 21, paragraph 3.17, page 16.

⁸⁵ Akahori, H., Karmali, V., Polavarapu, R., Lyle, A.N., Weiss, D., Shin, E., Husain, A., Naqvi, N., Van Dam, R., Habib, A. and Choi, C.U., 2015. CD163 interacts with TWEAK to regulate tissue regeneration after ischaemic injury. *Nature communications*, 6(1), p.7792.

⁸⁶ Fujiwara, Y., Ohnishi, K., Hortlad, H., Saito, Y., Shiraishi, D., Takeya, H., Yoshii, D., Kaieda, S., Hoshino, T. and Komohara, Y., 2020. CD163 deficiency facilitates lipopolysaccharide-induced inflammatory responses and endotoxin shock in mice. *Clinical & translational immunology*, 9(9), p.e1162.

proportionate and informed by the specific details of the targeting projects in all cases.

Section 4

11. Do you have any additional comments for AWC on this topic that have not already been covered in the questions above?

- Precision breeding methods (e.g. based on genome editing) offer many opportunities, such as the **control of infectious disease** in livestock, including zoonotic ones, but also will require careful risk management. We discussed the theme “**genome editing approaches for zoonotic resistance**” at ASM 2023. Genome editing approaches present similar challenges to other disease control strategies, such as the use of drugs or vaccination. Management of infectious diseases often requires holistic and One Health approaches. There will always be an evolutionary arms-race between pathogen and host and selective pressure acting on the mechanisms of infection and resistance^{87,88}. GE approaches, even if initially successful in controlling a pathogen, will need to be accompanied by robust and continuing epidemiological monitoring of emerging biological risks.
- When you develop resistant animals and reintroduce them to future variants of the pathogen, there could still be biosecurity risks. In the research phase for the PRRS resistant pigs (see answer to question 9), researchers pushed the accelerated evolution of the virus to probe this biosecurity risk. Similarly, the recent publication of **avian influenza resistant GE chicken** exemplifies the need to challenge the edited animals with higher doses of the virus, above the natural load to which they become resistant. As noted in the paper, following direct inoculation with a higher dose of the virus, scientists observed: “breakthrough infection occurred in the GE birds. Influenza virus is notorious for its ability to evolve, and we detected a series of different amino acid substitutions in the viral polymerase genes of viruses isolated from the GE chickens that had enabled adaptation of the enzyme to co-opt support from the edited ANP32A protein, and also to utilise otherwise suboptimal ANP32 family members. These mutations unexpectedly allowed the usually host-restricted avian influenza polymerase to use the shorter human ANP32A and B and thus partially adapted the viral polymerase for replication in mammals. Although unintended, this consequence clearly indicates the importance of a robust genome editing strategy and **subsequent appraisal that includes challenge with multiple avian influenza genotypes at non-physiological exposure levels to rule out the opportunity for adaptive viral evolution**”⁸⁹.
- The identification of GE targets for resistance should be carefully considered and stress-tested in the lab, particularly if it involves orthologues of pathogen host factors. In our submission to the DEFRA consultation in 2021 we wrote that: “genome editing of host factors (e.g. cellular receptors) to make livestock

⁸⁷ Sanderson, T., Hisner, R., Donovan-Banfield, I.A., Hartman, H., Løchen, A., Peacock, T.P. and Ruis, C., 2023. A molnupiravir-associated mutational signature in global SARS-CoV-2 genomes. *Nature*, 623(7987), pp.594-600.

⁸⁸ Read, A.F., Baigent, S.J., Powers, C., Kgosana, L.B., Blackwell, L., Smith, L.P., Kennedy, D.A., Walkden-Brown, S.W. and Nair, V.K., 2015. Imperfect vaccination can enhance the transmission of highly virulent pathogens. *PLoS biology*, 13(7), p.e1002198.

⁸⁹ Idoko-Akoh, A., Goldhill, D.H., Sheppard, C.M., Bialy, D., Quantrill, J.L., Sukhova, K., Brown, J.C., Richardson, S., Campbell, C., Taylor, L. and Sherman, A., 2023. Creating resistance to avian influenza infection through genome editing of the ANP32 gene family. *Nature Communications*, 14(1), p.6136.

animals resistant to a zoonotic disease may require a carefully thought-through regulatory approval pathway from proof of concept stage to commercial release, via progressive stages of biocontainment, intermediate small scale releases and longer term monitoring/surveillance of emerging impacts. For example, scientists are studying species-specific susceptibility factors to influenza A viruses,⁹⁰ some of which are adapted to humans and have pandemic potential. Genome editing could offer ways to introduce resistance to avian-specific viruses in farmed chicken to alleviate the poultry sector of an enormous health and welfare challenge. However, GE targeted host factors could be conserved between birds and humans therefore introducing the risk of driving the evolution of influenza towards a form that is more likely to infect humans and other mammals. [...] Tackling avian influenza in poultry can benefit the animals and also reduce the risk of the emergence of human-adapted viruses if the use of genetics and other practices is appropriately managed and balanced". We concur with Idoko-Akoh et al that: "future assessment of GE animals, after the research phase of their development and prior to their distribution, should take into account whether appropriate investigatory steps have been carried out to evaluate if genome-edited livestock might drive pathogen evolution. This is especially relevant for pathogens with zoonotic potential as was shown here. We suggest that a suitable strategy for generating avian influenza resistant chickens will require multiple edits that destroy the proviral potential of ANP32A, B and E to eliminate the likelihood that escape mutants can arise"⁹¹.

- At the Animal Science Meeting 2023, workshop participants discussing "animal breeding in the genomics era", identified the need for increased **public understanding of farming and food production**, in order for technological advances in animal breeding to be properly contextualised and judged. This improvement should also apply to public understanding of animal welfare standards on farm and assurance schemes.
- On **data-sharing and transparency**: one observation raised during the same workshop related to the increased need for openness in data sharing between the breeding and animal welfare research communities. Researchers have previously found it hard to access extensive and good quality data to independently assess impacts of breeding strategies on the health and welfare of the animals. The conversation and potential agreement on data sharing should be conducted in a way that safeguards intellectual property and commercially sensitive information.
- A workshop participant suggested that information about the welfare of GE animals authorised for marketing under the Act should be made available, as it should be considered a public good. This point resonates with key recommendations 6 and 7⁹² of the Nuffield Council on Bioethics report on

⁹⁰ Long, J.S., Idoko-Akoh, A., Mistry, B., Goldhill, D., Staller, E., Schreyer, J., Ross, C., Goodbourn, S., Shelton, H., Skinner, M.A. and Sang, H., 2019. Species specific differences in use of ANP32 proteins by influenza A virus. *Elife*, 8, p.e45066.

⁹¹ See ref 90

⁹² Recommendation 6: We recommend that the use of breeding indices that reflect a profile of heritable characteristics, including those that are of public or social as well as economic value should be explored as a possible regulatory tool. Commercial breed developers placing animals or animal reproductive materials on the market could be required to publish these indices.

Recommendation 7: We recommend that an appropriate, independent, and trustworthy body (identified or established by Defra in the UK) should monitor the longitudinal development of breeding lines (e.g., in the dimensions captured by enhanced breeding indices – see recommendation 6). This body should report on these matters to the public authority or authorities

Genome Editing in Farmed Animal Breeding, which focus on reliable, comparable and accessible data on the effects of breeding practices to support governance of the sector, animal welfare assurance and adherence with societal values.

- On the **establishment of regulatory fora**: ideally, advances in knowledge and technological application in different species would be best communicated by animal scientists and breeders directly to regulators & policymakers through stakeholder fora, in which the relevant DEFRA teams would gather views and test upcoming policy/guidance documents with developers and end-users. The RSB supports the secretariat of the UK Bioscience Sector Coalition⁹³, which is the main animal research sector representative body in discussion with the Home Office Animals in Science Regulation Unit. We would welcome involvement in follow-up conversations with DEFRA about the establishment of any stakeholder forum where criteria and approaches for animal welfare assessment of novel breeding technologies are proposed and developed.
- As part of this review, the AWC may wish to collect useful examples of **industry standards**, such as Red Tractor, European Chicken Commitment or the RSPA assurance system, which may inform the development of the data requirements for the animal welfare assessment under the GT(PB)A 2023.
- The choice of **measurable variables to construct animal welfare indices** must be meaningful, i.e. you should avoid the case where most variables do not show any effect/difference between control and GE animals. Indices should include hypothesis-driven testing of variables specific for a trait/project but also a number of basic welfare variables that are assessed more holistically across cases. Data collection requirements under the new Act should be aligned with global regulatory/breeding context. Data requirements should be proportionate and adaptive to the current breeding context and the specific aims of the novel breeding project. For example, researchers might work with small populations of rare breeds or carry out proof-of-concept experiments in few animals, where certain effects may not be measurable. Data acquisition protocols, part of future regulatory requirements for the development and commercial phase, would need to make statistical sense and be robust. Regulators will need to think about the need for power calculations to estimate the number of animals that need to be assessed to have a realistic chance of detecting a significant difference between the GE and control populations. If the effect is quite small, or there is high variability in whatever is being measured, then no difference will be found if the group size is insufficient.
- On the importance of **proportionality in future regulations**: the potential outcomes and benefits for using functional genome annotation, genotype-to-phenotype information and genome editing in animal breeding programmes must be understood and assessed in comparison with current or past breeding practices. Some technological interventions may simply complement current practices instead of supplanting them completely. Therefore, the assessment

having oversight of farmed animal breeding (in the UK, the Animals in Science Committee, the Animal Welfare Committee, the Animal and Plant Health Agency and/or the proposed Animal Sentience Committee, as the case may be – see recommendation 12). The body should ideally have access to information to enable the validation of breeding effects, provided in confidence if necessary, and advise where information is lacking. We encourage breeders to facilitate scientific research using their data, leading to publication in peer-reviewed journals. <https://www.nuffieldbioethics.org/assets/pdfs/Genome-editing-and-farmed-animal-breeding-FINAL-WEB-PDF.pdf>

⁹³ <https://www.rsb.org.uk/policy/groups-and-committees/uk-bioscience-sector-coalition>

of the benefits/impacts of emerging biotechnologies should be contextualised and proportionate.

Appendix 1

Category	Trait	Species	Editing target
Health	PRRSV resistance	Pig	<i>CD163</i>
	ASFV resilience	Pig	<i>RELA</i>
	IPN resistance	Atlantic Salmon	<i>nae1</i>
	Bovine tuberculosis resilience	Cattle	<i>SLC11A1 (NRAMP1)</i>
Welfare	Polledness (Hornlessness)	Cattle	<i>P_c POLLED</i>
	Heat tolerance (Coat color)	Cattle	<i>PMEL</i>
	Heat tolerance ('Slick' coat)	Cattle	<i>PRLR</i>
Reproduction	Sterility/surrogate broodstock	Atlantic Salmon	<i>dnd</i>
	Sterility/surrogate sires	Pig	<i>NANOS2</i>
		Goat	
		Cattle	
Sterility/surrogate hosts	Chicken	<i>DDX4 (Vasa)</i>	
Appearance	Plumage color (Dominant white)	Chicken	<i>PMEL17</i>
	Feather type (Frizzled)	Chicken	<i>KRT75</i>
Production	Enhanced muscle growth	Cattle	<i>MSTN (GDF8)</i>
		Sheep	
		Goat	
		Pig	
		Red Sea Bream	
		Channel Catfish	
	Hair fibre length	Goat	<i>FGF5</i>

Table: Examples of genome editing in farmed animal species to improve five different categories of trait.

From Clark, E 2022, Breeding in an Era of Genome Editing. in Encyclopedia of Sustainability Science and Technology. Springer Nature. https://doi.org/10.1007/978-1-4939-2493-6_1122-1

Appendix 2

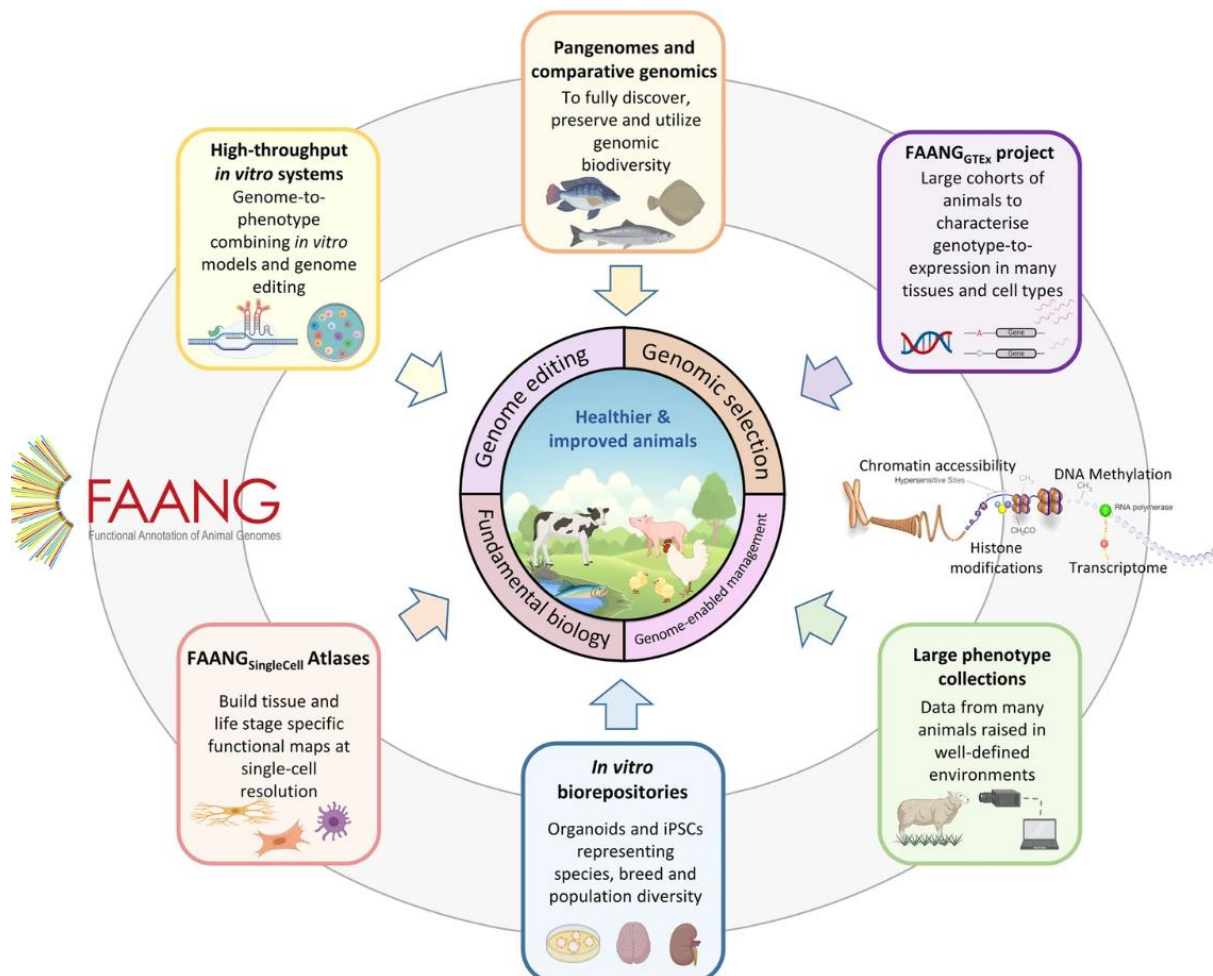


Figure: Priorities for the next decade of Functional Annotation of Animal Genomes (FAANG) research project

Clark, E.L., Archibald, A.L., Daetwyler, H.D. et al. From FAANG to fork: application of highly annotated genomes to improve farmed animal production. *Genome Biol* 21, 285 (2020). <https://doi.org/10.1186/s13059-020-02197-8>

Appendix 3

Some considerations about targeting CD163 via genome editing and its wider physiological roles:

In pigs, the deletion of domain 5 as opposed to the whole gene preserves critical functionalities of the CD163 protein, particularly those associated with the function of the soluble CD163, as a master orchestrator of the inflammatory response. CD163 is cleaved off from macrophages through a regulated process that induces ADAM17-mediated cleavage of the protein from the surface, generating the soluble version of the protein (sCD163). The functional entities that orchestrate the inflammation response are so-called TWEAK, TNF-like weak inducer of apoptosis, signals. Interestingly, these signals are located on all the scavenger receptor cysteine-rich (SRCR) domains of CD163, with the exception of SRCR5, which is the domain removed in the edited pigs and in the pigs taken forward to commercialisation.

In the aforementioned papers, describing the development of GE pigs, both Burkard *et al.* PloS Path 2017, and Burkard *et al.* JVI 2018, looked at the hemoglobin/haptoglobin scavenging activity of CD163, which was unaltered following deletion of domain 5. The scavenging activity is associated with the early SRCR functions, which are also responsible for the uptake and presentation of *Staphylococcus aureus*. One could therefore assume that the uptake of *S. aureus* would be intact in the GE animals because no changes were made in CD163 protein domains important for both the scavenging function and its antibacterial function.

The authors also measured and confirmed the presence of sCD163 in both edited and wild type animals. Given that the genome editing strategy targeted only domain 5, the scientists did not expect any functional changes to the TWEAK signals and therefore assumed that the orchestration function of the edited protein was still intact. Furthermore, the group observed that one pig in connection with standard husbandry developed a *Lawsonia intracellularis* infection. This pig showed significantly elevated soluble CD163 levels, showing that the functional production of the soluble protein is intact [personal communication as this was not characterised in the paper].

In relation to the role of CD163 in response to ischaemic injury, it is the soluble form of CD163 that orchestrates the local response at the site of injury by binding and deactivation of TWEAK molecules. Soluble CD163 and TWEAK signalling are maintained in the domain 5 deletion pigs so one could assume that muscle regeneration in response to injury should not be altered in the edited pigs.

The 2023 Animal Science Meeting Report

The RSB annual Animal Science Meeting (ASM 2023) took place in central London and focused on research and policy developments concerning the use of **genetic technologies and precision breeding in animal science**.

The meeting is organised and supported by the RSB Animal Science Group, a special interest group of the Royal Society of Biology, which brings together a broad spectrum of UK bodies that are actively involved in supporting, funding, or formulating policy on animal research. The whole-day meeting was chaired by Professor Clare Stanford FRSB, Chair of the Animal Science Group.

This year's theme was selected in light of the new [Genetic Technologies \(Precision Breeding\) Act 2023](#) – GT(PB)A 2023 – and forthcoming regulations for the use of genome editing and precision breeding in farmed animals. The theme is also aligned with the RSB science policy priorities for 2022-2027, which include a focus on enabling appropriate regulation and societal debate on the use of genetic knowledge and technologies in ethical biological innovation.

The meeting brought together a variety of sectors including: academia, industry, funding bodies, animal welfare organisations, learned societies and professional associations. Representatives from several regulatory and policy teams across the public sector also joined the event.

The meeting consisted of five talks on scientific developments in the field and associated ethical issues, followed by an afternoon session of four parallel workshops. These were facilitated by experts and sector leaders, including members and Fellows of the Royal Society of Biology.

Expert talks:

The **first talk** of the day was given by **Dr Gareth Arnott** from Queen's University Belfast, who presented preliminary results of a systematic literature review examining the **welfare implications of animal breeding and breeding technologies in commercial agriculture**.

The aim of this project is to analyse scientific literature published since 2011 on the topics of animal breeding, breeding technologies and welfare of farmed animals. The results will update the content and recommendations of the 2012 Farm Animal Welfare Committee (FAWC) report, '[Opinion on the welfare implications of breeding and breeding technologies in commercial livestock agriculture](#)' and highlight knowledge gaps and areas where more evidence is needed. The literature review includes papers on a number of species, such as: beef cattle, dairy cattle, sheep, pigs, broiler chickens, laying hens, turkeys and salmon. Dr Arnott described the detailed process used to select peer-reviewed literature and additional sources, with expert additions also accepted from a range of stakeholders. Results from the literature search were classified using the PRISMA guidelines⁹⁴ and were quality-controlled through a set of filters, including the application of appropriate research standards based on the REFLECT statement⁹⁵.

Dr Arnott summarised general facts and statistics of the selected studies in the dataset, including the species of animals and the general themes of the papers, such as: genetic analysis and mapping, breeding and selection, and reproductive technologies. The dataset also includes association studies of traits that could improve welfare, for example: looking at disease resistance; prevention of congenital conditions; parasite resistance; polledness in sheep and cattle and the genetic basis for behaviour.

Dr Arnott presented an example of their research approach by looking at the breeding of turkeys as a case study. The papers included in the analysis focused mainly on productivity

⁹⁴ <http://www.prisma-statement.org/>

⁹⁵ Sargeant, J.M., O'connor, A.M., Gardner, I.A., Dickson, J.S., Torrence, M.E., Dohoo, C.M.P.I., Lefebvre, S.L., Morley, P.S., Ramirez, A. and Snedeker, K., 2010. The REFLECT statement: reporting guidelines for randomized controlled trials in livestock and food safety: explanation and elaboration. *Journal of food protection*, 73(3), pp.579-603.

traits, with a smaller subset dedicated to health and welfare traits. However, the entire dataset offers a wider range of approaches. Despite the preliminary nature of this project, one of the take-home messages of the talk was the importance of taking into account animal welfare in the published literature and how future research can fill in any emerging evidence gaps, particularly given the importance of animal welfare as a social license when applying genetic technologies to farming and animal breeding.

The next stage of the analysis is to interrogate the content of the papers. While a quantitative meta-analysis is useful in evaluating medical studies, a narrative approach will be used to examine the findings of this project, given its broad scope and range of expected animal welfare outcomes

The **second talk** was given by **Dr Emily Clark** from the Roslin Institute in Edinburgh and discussed how **advances in genomics, functional annotation and potential uses of genome editing** are likely to be applied in **future farmed animal breeding**. Sustainable improvements in the efficient production of farmed animals will be needed in coming decades to provide healthy food for a rapidly growing human population. The challenge is to produce more food using fewer resources, in a sustainable way that meets societal expectations, improves animal health and welfare and mitigates the effects of rapidly changing climates.

Chief amongst the improvements required in farmed animal breeding is the more accurate use of an animal's genotype to predict its phenotype. Advances in the analysis of genome function will provide tools and knowledge to answer the genotype-to-phenotype question. The [Functional Annotation of Animal Genomes \(FAANG\) project](#) is an international effort to characterise the functional elements of the genomes of farmed animals.

The first stages of FAANG focused on foundational data generation to characterise expressed and regulatory genomic regions, curation and provision of highly annotated farmed animal genomes. These were largely based on high depth approaches at the individual level. The primary challenge facing this community now is harnessing both the resources generated, and the potential of new technologies, to link genotype, phenotype and genetic merit in order to translate this research from the laboratory into industrial application in the field. For example, genome editing represents a potential major route for the application of FAANG data in farmed animal breeding, via detection and utilisation of causative variants that affect important traits, 'introgression-by-editing' of favourable naturally occurring alleles into a closed breeding population, and/or creation of *de novo* alleles with desirable effects. New digital and artificial intelligence technologies also offer the potential to monitor phenotypic variation in traits that affect animal health or welfare, over multiple generations and at a farm level. Continued public investment, international collaboration, data infrastructure and training of new scientists are now needed to increase the potential application of genotype-to-phenotype research in animal breeding even further.

The **third presentation** was given by **Dr Andy Greenfield FRSB** from the University of Oxford who talked about the **ethics of genome editing and farmed animal breeding**. CRISPR/Cas genome editing has been available for just over a decade and its impact on the biological and medical sciences has been profound. Its development has meant that any genome is potentially an experimental genome and so any animal can be an experimental animal. But, whilst genome editing has clearly influenced research using animals, with attendant ethical concerns, it is arguably the use of genome editing in animals beyond the research arena that has captured the public imagination: for instance, gene drives to control wild populations of insects or rodents; extensive editing of the pig genome to support xenotransplantation; and the use of gene editing in agriculture and food production.

The last of these is an especially sensitive topic, whether the focus is on plants or animals. The central question of the talk was whether and how genome editing should contribute to the ethical farming of animals. Dr Greenfield surveyed some of the arguments that have been made concerning the ethical acceptability of incorporating genome editing into animal breeding for farming. He suggested that we should not be distracted by dystopian visions of

the future of farming, but instead consider how ethical considerations impinge on our assessment of current breeding regimes for farmed animals, as science and technological applications evolve. Which trajectories should we adopt now? He referred extensively to the report on social and ethical issues arising from [genome editing and farmed animal breeding by the Nuffield Council on Bioethics \(2021\)](#) as an organising framework to explore certain issues, including ‘the good life’ of animals and technological exceptionalism.

The **fourth talk** was given by **Dr Thomas Peacock**, fellow and lecturer at the Pirbright Institute and Imperial College London, on the topic of **genomics and genome editing for zoonotic disease resistance**. As exemplified by recent avian influenza virus, African swine fever and Bluetongue outbreaks, infectious diseases of animals can cause huge economic damage and threaten global food security. Vaccines are widely used to control these diseases but, even when available, can sometimes have severe constraints or limited effectiveness. As a consequence, gene editing for disease resistance in livestock has emerged as a promising alternative technology. This approach, like vaccines, can have the added benefit of reducing zoonotic disease burden by limiting human exposure to zoonotic viruses.

In his talk, Dr Peacock explored the benefits, limitations and potential issues with current strategies for gene editing of animals to reduce zoonotic burden, with a particular focus on influenza virus. He explored the potential impact of gene editing on virus evolution and how rigorous testing of these systems in the laboratory can help us both better understand the safety of these systems, as well as shine new light on basic virus biology.

The **final scientific talk** was given by **Dr James Turner**, principal group leader & assistant research director at the Francis Crick Institute, on the topic of **gene editing for single-sex litters**. In mice and humans, females and males have the same autosome complement but differ with respect to their sex chromosomes: females are XX, while males are XY. In scientific research and global resources in agriculture, a single sex is often required in surplus. The ethical and financial burden of producing and culling animals of the undesired sex is considerable. In his talk, Dr Turner outlined how researchers have used CRISPR-Cas9 technology to generate single-sex litters, using the mouse as a model system. He described the challenges that remain in applying this technology to agriculture, and outlined public perceptions towards this use of the technology.

Workshop discussions:

In the afternoon, meeting participants took part in four parallel workshops led by sector leaders and members of the Royal Society of Biology on the following themes:

1. **Assessing animal welfare impacts of precision breeding**
2. **Animal breeding in the genomics/genome editing era**
3. **Public perceptions of genome editing in farmed animals**
4. **Genome editing approaches for zoonotic resistance: opportunities and challenges**

Here we summarise the main take-home messages from each roundtable, which will inform upcoming policy and communication work by the Royal Society of Biology on the use of genetic technologies in animals.

1. Assessing animal welfare impacts of precision breeding

- a. The conversation about how we measure the impacts of genome editing on the welfare of animals extends beyond the animal welfare sectors. It will be affected by politics, policy, trade, food security, etc.
- b. We should be assessing welfare indices that include both specific and broad measures.

- c. In the phase of regulation under the Animals (Scientific Procedures) Act, there could be a large number of indices that are considered risk factors for animal welfare and need to be measured during the experimental phase. The number of indices adapted for use at farm level might differ from those used in the research phase. They will need to be developed and agreed in a context dependent manner.
- d. The 3Rs concept (Replacement, Reduction and Refinement) should be applied to genome editing across farmed animal species.
- e. There is also need for a harm versus benefit analysis concept in the context of genome editing in farmed animals.

2. Animal breeding in the genomics/genome editing era

- a. There is need for non-specialist audiences to understand how farmed animal breeding operates today. Communication strategies should explain the context for the use of genomics and genome editing in farming. Various methodologies and processes take place in traditional versus novel breeding scenarios: a spectrum of technological interventions exists.
- b. The group discussed outcomes of breeding programmes: traits, welfare outcomes and the context of the species/productions systems are all important factors to consider. However, outcomes depend on the choice of indices: what and how we decide to measure and under what conditions.
- c. There is need for better transparency of breeding data and its accessibility for animal welfare researchers (and other stakeholders). Specifically, this should apply to how breeders share data that are relevant to animal welfare. The Animal Welfare Declaration in the new Act provides an opportunity to improve on the *status quo*.
- d. Where is the main focus of regulations? We should aim to reach a level-playing field and proportionality for the animal welfare assessment, with respect to how current breeding processes are regulated. The GT(PB)A 2023 sets the stage for new regulations but we need to be targeted in the requirements. There should be hypothesis-driven testing and holistic assessment of basic welfare variables. There should be alignment between the global regulatory/breeding context and the need for data collection under the new Act.

3. Public perceptions of genome editing in farmed animals

- a. Engagement should be an ongoing effort because current events can change public opinions.
- b. We need to marry up stated views of citizens with the behaviour of consumers, as these can differ.
- c. People are unaware of our food system in general. They are often not familiar with farming and food production methods, even when they do not involve advanced technologies or genome editing.
- d. Language is fundamental: the choice of wording affects people's responses.
- e. Can policy define the acceptable purposes for the use of genome editing? Is this the responsibility of the Animal Welfare Committee under the new Act? Who does define what a benefit is? And for whom? Where is the transparent assessment of benefits in the policy process?

4. Genome editing approaches for zoonotic resistance

- a. The current context of antimicrobial use and resistance (AMR) makes genome editing solutions attractive.

- b. Tackling vector-borne diseases provides additional appeal for the use of genome editing approaches, but this is more of an intermediate target.
- c. What is the additional risk of genome editing versus existing solutions? The timescale of how these methods help us manage disease is an important factor to consider.
- d. There is no silver bullet. We need a toolbox of approaches for different situations or to use different technologies in combination (and with different timescales of application).
- e. There is need for additional regulatory approval, scrutiny and policy if genome editing is used for zoonotic versus more restricted types of infections.
- f. There is also need for international alignment of regulations in this area, as well as openness and sharing of research data.