REVIEW OF CURRENT AND NEW TECHNOLOGIES RELEVANT TO FAnGR

Introduction

There is a range of technologies that can have a role in the breeding of farm animals and therefore impact on genetic resources, although their relevance may vary depending on whether they are applied to breeding and sustainable use of mainstream breeds or the conservation of breeds at risk. There will be continued developments in scientific knowledge and increased understanding over time which will lead to refinements to existing technologies as well as the development of new technologies. This horizon-scanning report, prepared by its Farm Animals Genetic Resources Committee (FAnGR) at the request of Defra, reviews the current situation with respect to the relevance of these various technologies in animal breeding. In reviewing them it is important to highlight that they are all tools that have the potential to be very helpful, but only if used appropriately. Inappropriate use could equally threaten the maintenance of farm animal genetic resources (*e.g.* it could result in high levels of inbreeding or proliferation of undesirable genetic conditions) in both mainstream breeds and breeds at risk.

Reproductive technologies

Included within this category are artificial insemination (AI), semen freezing, semen sexing, multiple ovulation and embryo transfer (MOET), freezing of embryos and ova, and *in vitro* fertilisation. Some of these (AI, semen and embryo freezing, MOET) are in widespread use in mainstream animal breeding, but their success and application varies across the species. For example, the use of frozen semen is very successful in cattle, but in other species such as sheep, goats, pigs and horses pregnancy rates are significantly lower with frozen semen, with the result that fresh/chilled semen is often the method of choice for conventional breeding purposes. Nevertheless, even in these species, semen freezing can have a significant role in breed conservation (see section on Bio-banking). In addition, the ease with which AI can be carried out in different species does vary, and this partly explains differences in adoption rates. For example, in cattle, goats and pigs AI is trans-cervical, allowing semen to be deposited directly into the uterine lumen. However, in sheep this is not yet possible, requiring a laparoscopic approach through the abdominal wall to permit deposition of semen into the uterine lumen which is essential to generate acceptable conception rates with frozen semen. Development of techniques that would allow effective trans-cervical AI in sheep could dramatically increase the benefits from its use in mainstream and breeds at risk.

The application of MOET is both successful and of far greater importance in ruminant species than in pigs because of their much lower litter size. However, the induction of multiple ovulations in horses is more problematic, because of the different structure of their ovaries. Across all species, the ability to successfully freeze embryos is not only useful for the international movement of genetic resources in mainstream breeds but can also play a significant role in breed conservation (see section on bio-banking). When combined with performance recording to identify superior genotypes, these reproductive technologies can have a significant benefit for increasing rates of genetic improvement for production efficiency, and this benefit underpins their widespread use in mainstream breeds.

Genomic selection and related techniques

DNA markers

Naturally occurring, spontaneous mutations to create so called polymorphisms occur in the DNA of all animal species. Indeed, it is these polymorphisms that generate genetic variation within breed populations and differences between breeds. Historically, selection and breed creation would have been done without knowledge of which polymorphisms were being positively selected, but recent advances in genomic tools now offer a means to better understand and manage the process.

The main focus initially was on developing individual genomic tests for traits of commercial interest, and a number of commercially available DNA marker test have been developed (see http://hccmpw.org.uk/publications/breed_improvement/ '*Genetic markers in beef and sheep breeding programmes: Unravelling the practicalities of DNA technologies*). However, as further advances have been made it has allowed more detailed consideration to be given to most, if not all, the DNA that individual animals carry. Following rapid reductions in the cost of using approaches such as genome sequencing, and development of advanced analytical approaches such as genome wide selection, it is now possible to use the large amount of data generated to increase the effectiveness of modern breeding programmes in mainstream breeds and breeds at risk. Potential benefits would also be expected to increase as further advances are made with genomic tools, reductions in cost and analytical approaches.

While the genomic tools available can be used to help increase the effectiveness of breeding programmes aimed at improving commercial performance (which is the main focus of the genomic selection approach: see below), they can also have an important role in managing inherited recessive diseases that can arise (*e.g.* micro-opthalmia in sheep, spider lamb syndrome, neuropathic hydrocephalus in calves). Such inherited diseases can exist within both mainstream breeds and breeds at risk, and, where they are present within a breeding population, the ability to distinguish between heterozygous carriers and homozygous non-carriers is crucial. Such stand-alone DNA tests which provide this ability can assist breeders in managing their breeding programmes to (i) avoid the production of 'double copy' individuals with the condition and (ii) the eventual elimination of all carrier animals from the breeding population. The Ridgene Manual (see http://www.signetfbc.co.uk/wp-content/uploads/2014/11/ridgene_manual_digital.pdf provides detailed guidance in how this can be achieved. As such they can have relevance to both mainstream breeds and breeds at risk.

As well as being used for selection purposes, SNP analysis is widely used to define breeds and their relatedness. It also, offers a means to more accurately (compared to analysis of pedigree alone) assess the relatedness of individuals within a given population, as well as the level of genetic diversity within breed and species. Using SNPs in this manner involves comparison of the SNP profile across individuals within the population. Such an approach could be especially important for the conservation of rare breeds/breeds at risk by providing breeders with a technology to monitor and maintain genetic diversity within them.

Genomic selection

New genomic marker technologies can now provide detailed analysis of a sample of single nucleotide polymorphisms (SNPs) evenly spread across the **whole** genome of individual animals relatively quickly. Unlike with specific gene DNA marker tests (see above), genomic selection relies on statistically associating traits of interest with specific SNPs using a fairly large 'training population' (~1500-2000 animals). Subsequently, the most appropriate combination of SNPs rather than individual animal performance is used as the basis for selecting the most desirable animals. With use of appropriate 'training' populations and trait measurement, this technique provides opportunities to select for 'difficult to measure traits' such as robustness and disease resistance far more precisely than with conventional breeding, as well as being used for selection for more conventional traits.

Genome wide selection is generally being adopted where large populations of a breed exist and the commercial advantage through accelerating the rate of genetic improvement can justify the increased costs of implementation, as is the case in some mainstream breeds of cattle, pigs and poultry. This is because the costs of generating the required information using 'training populations' are currently prohibitive except for species and populations that are fully or partly controlled by multinational companies where returns in sales of breeding stock/semen can justify the costs. Genomic selection is already having an impact in Holstein dairy cattle where it can dramatically increase rates of genetic progress by allowing selection to take place in young animals rather than having to wait for the results of their progeny tests. However, as with all breeding technologies, it is essential that genomic selection does not narrow the genetic base and reduce effective population size. Within that context, it is important to realise that an added advantage of using genomic markers is that it can be used to unequivocally define parentage, and therefore may be more accurate than pedigree recording.

Because of the high costs of establishing 'training populations' and the high numbers of animals required for them, genomic selection is beyond the reach of small breeds (including many mainstream sheep and beef cattle breeds) and especially breeds at risk. Even with further developments in genome sequencing, it is unlikely that the costs will reduce sufficiently to overcome this limitation. However, consideration is being given to whether 'multi-breed training populations' could provide a cost-effective route for their application in at least some of these breeds, and further advances in this area may widen the number of breeds and species that could feasibly consider using the approach.

Advances in statistical genetics techniques

Of particular interest in some species (*e.g.* pigs, chickens) is the potential for selection based on groups rather than individual animals. Such an approach could be used to select animals that perform well when kept in groups, rather than basing selection on individual animal performance, to reduce aggressive behaviour at feeding, leading to more uniform performance of animals within the whole population. This requires a different statistical approach compared with conventional selection.

The use of genomic techniques is resulting in a large amount of data being generated for animal populations, which is likely to increase even further as sequencing costs continue to reduce and knowledge of the function of different parts of the genome increases. More advanced statistical models will be required to allow effective use of such data.

The wide range of genomic tools also allows consideration to be given to developing advanced statistical approaches that allow a high level of benefit to be achieved without the need to fully sequence the genomes of all the animals in the populations (*e.g.* through imputation).

Genome editing

A very new technology currently being developed and further refined is the novel molecular biology tool for the fine-scale editing of individual genes within existing genomes of an embryo to bring about desirable genetic change. Such modifications can be as small as changing an individual nucleotide base within a specific gene of the embryo which is then indistinguishable from another naturally occurring polymorphism within a given breed or species. The technology is generating considerable interest within human medicine because of its potential for the elimination of inherited diseases (due to a naturally occurring mutation) from specific individuals within a population. The technology could also offer the same benefit in animals, particularly where inherited diseases arise in breeds at risk and where effective population size is already at dangerously low levels.

The technology also offers the means to generate new variation within a given breed that could be used to accelerate the rate of genetic improvement for traits of commercial interest. However, it is unlikely to be attractive for most traits because of the high costs and time required to generate a sufficient number of animals, particularly where good progress is already being achieved using genomic selection. Nevertheless, it may be an attractive option to introduce variation known to

confer resistance to specific diseases where current breed populations have little or no resistance. One such example under investigation is the introduction of natural resistance to African Swine Fever virus in the pig. Very recently, American scientists have used this technology to experimentally produce pigs that are immune to the porcine reproductive and respiratory syndrome virus (PRRSV) (see Nature Biotechnology 2015; http://dx.doi.org/10.1038/nbt.3434). Genome editing may also make it possible to edit the structure of the polling gene in those cattle breeds that are naturally horned, thereby improving animal welfare, without the need for initial crossbreeding to introduce the polled variant of the gene in question.

Currently this technology is very expensive, and there is an urgent need to generate more exemplars within farm animals to provide unequivocal proof of its potential in such circumstances. It is possible that once such exemplars exist its application could be relevant to both mainstream breeds and rare breeds, albeit that the traits of interest might be different. It is also essential to recognise that gene editing is simply changing the structure of a specific gene from one naturally occurring polymorphism to another: unlike transgenic GM technology, it does **not** involve the introduction of 'foreign' genes from another species. Making clear such a distinction from the outset will be critical for public acceptability of the technology.

Bio-banking and related cryopreservation technologies

As already indicated in an earlier section, reproductive technologies can have a significant role in bio-banking, and if appropriately applied can have special relevance for the conservation of rare breeds/breeds at risk to safeguard against population losses due to unforeseen events that could threaten their survival. Existing techniques for the cryopreservation of semen, embryos, ova and somatic cells (for cloning – see later section), as well as avian primordial germ cells (see later section), could all have a significant role in breed conservation. While in some species pregnancy rates following use of frozen semen are lower than for fresh chilled semen (see section on reproductive technologies), this difference is not sufficient to preclude the use of frozen semen for bio-banking purposes. In those species where MOET is successful and/or where the freezing of semen and embryos is possible (cattle, sheep, goats, pigs), these are the preferred tissues for bio-banking especially for breed conservation purposes because *in vitro* fertilisation using cryopreserved ova to generate embryos for transfer into recipient females is less successful than using *in vivo* derived embryos and can result in welfare problems because of foetal oversize. In spite of this, and where the bio-banking of embryos has not been possible, cryopreservation of ova can provide an additional safeguard against the loss of rare breeds/breeds at risk.

In laying down cryopreserved semen and embryos (or ova) in a bio-bank it is important to make sure that these are representative of as broad a genetic base of animals as possible within the breed to maintain genetic diversity. Current *ex situ* stores from breeds at risk appear to be largely limited to frozen semen, which means that regeneration of the breed in the event of a major loss of *in situ* populations would be impossible without the introduction of crossbreeding followed by backcrossing. While the crossbreeding and backcrossing approach may be appropriate when all else fails, it is important to realise that this will inevitably result in the introduction of genetic material from the 'alternative' breed used in the initial crossbreeding process. Conservation organisations therefore need to give careful thought to the cryopreservation of embryos as well as semen to guard against this eventuality, especially now that the costs associated with generating and storing embryos has fallen. It is also important to recognise that *ex situ* stores of semen laid down for other purposes (*e.g.* as part of the National Scrapie Plan for sheep) have significant limitations for breed conservation purposes and are not an appropriate substitute for carefully planned bio-banks of genetic material.

While bio-banking of semen and embryos may be considered to be relevant only to rare breeds and breeds at risk, this is not necessarily the case. History has clearly shown that the relative importance of breeds to mainstream agriculture can change in a very short period of time, and that once important native breeds can quickly become native breeds at risk. To counter this possibility all breed societies should be encouraged to bio-bank genetic material for conservation purposes to guard against this eventuality. Such a strategy is especially important for some of the smaller mainstream breeds, especially those with limited geographical distribution which would also be very vulnerable to some unforeseen disease event leading to the loss of important genotypes.

Small breed societies should work closely with conservation organisations to seek the most cost-effective solution for bio-banking to guard against such eventualities.

Cloning

Cloning involves the removal of the nucleus from a somatic cell (any body tissue) of an animal and its transfer into an enucleated egg of a donor animal of the same species to generate an embryo for transfer into a surrogate mother. However, embryos derived through this process have a much lower success rate that those derived through conventional embryo transfer, and it may also result in significant welfare issues for a proportion of the resulting offspring (see FAnGR Report on Cloning for fuller details of the technique).

The main focus of commercial cloning in livestock breeding to date has been to multiply up high genetic merit animals. Indeed, it has been used in North and South America to create 'copies' of high genetic merit dairy bulls, with their semen being made available for conventional breeding purposes.

While there is considerable public resistance to cloning using somatic cell nuclear transfer in the UK, at least for mainstream breeds supplying food for human consumption, the cryopreservation of somatic cells from rare breeds/breeds at risk could be used through cloning to regenerate such breeds lost through some unforeseen catastrophic event (*e.g.* major disease culling) or to overcome 'bottlenecks' within a breeding programme by allowing animals that are unable to breed naturally (*e.g.* castrate males, aged animals) to generate progeny into the breeding programme. The use of cloning for the preservation of rare breeds does not have the same public resistance as cloning in mainstream breeds. However, while cloning does retain the nuclear DNA of such animals, a small proportion of the total DNA of an animal, the mitochondrial DNA, comes from the donor egg. As such, a clone is not a complete copy of the animal providing the somatic cell. Consequently, for those species in which cryopreservation of embryos is possible, this provides a more desirable approach for bio-banking since it retains all DNA, nuclear and mitochondrial, of the breed in question. This will result in the generation of a 'true' copy of the original donor breed and is therefore a more desirable option for breed preservation.

In the past cloning was a necessary step to facilitate genome editing but, as indicated earlier, recent advances with the genome editing approach mean that a cloning step is no longer needed to enable this.

Avian primordial germ cells

The preservation of poultry breeds at risk poses particular challenges, especially with respect to bio-banking, because of the very different structure of their ovum compared with mammals together with the current inability to successfully freeze poultry semen. Cloning using somatic cell nuclear transfer is also not an option, as it is in mammalian species, because embryo transfer is also not possible in avian species.

Recent developments in the chicken have indicated that the isolation and freezing of progenitor germ cells (PGCs) may provide an alternative approach for bio-banking in poultry. PGCs are specialised stem cells that can be isolated from Day 3 embryos and which, depending on the sex of the individual embryo, differentiate into those responsible for the production of either sperm or ova. Following isolation, these PGCs can be cultured and cryopreserved, and then transferred back into any Day 3 'recipient' chick embryo alongside its own PGCs. Some of the resulting gametes (ova and sperm) can thereby permit the regeneration of the breed from which the cultured PGCs were initially taken.

This technology is still in its infancy, but with further refinement, it offers for the first time exciting possibilities for the conservation of poultry breeds, allowing them to come into line with the various mammalian species. However, further research is required to identify ways to remove the recipient embryo PGCs, such that the resulting chick contains only the PGCs from the donor breed. In addition, current work has focussed largely on the chicken, and it will be necessary to establish whether, and under what conditions, this approach can be applied to all poultry species. While the

technology may have special relevance for the preservation of rare breeds and breeds at risk, it may also have a role in maintenance of important lines of mainstream poultry breeds used in commercial poultry production without the need to keep large populations of live birds.

Conclusions

There is a wide range of technologies that can have relevance in the breeding and preservation of both mainstream and rare breeds of livestock. Not all technologies have relevance to all situations, but the information provided has highlighted where each may have a role to play. In addition, an attempt has been made to provide guidance to breed societies and conservation organisations about the relevant merits and preferences of the different approaches. The challenge now is to convert these guidelines into actions where appropriate.