APPLICATION OF BIOTECHNOLOGY FOR THE GENETIC IMPROVEMENT OF LIVESTOCK: STATUS AND PROSPECTS

M. Georges
Department of Genetics, Faculty of Veterinary Medicine, University of Liège
20 Bd de Colonster, B-4000 Liège, Belgium

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Summary: Increasingly, biotechnology becomes an integral part of the arsenal of tools that can be used to improve animal production in order to meet the demands of the consumer within economic, environmental and ethical constraints imposed by society. Biotechnology contributes to animal production by improving the environmental component of the production systems as well as by improving the genetic make-up of livestock. After giving a general overview of biotechnology and animal production, this technical theme focuses on the three areas of biotechnology contributing to genetic improvement of livestock, i.e. reproductive biotechnology, livestock genomics and marker assisted selection (MAS), and transgenics. A questionnaire-based survey conducted amongst OIE Member Countries allowed us to briefly summarise the ongoing efforts in these areas. The questionnaire also aimed at defining the major challenges that will be faced by animal producers intending to incorporate biotechnology-based methods. Public distrust, lack of financial support for technology development in developing countries and questionable cost-effectiveness were identified as the major problems facing future implementation.

1. INTRODUCTION

Biotechnology per se has been an integral part of human culture for a very long time. Domestication of plant and animal species and the accompanying processes of artificial selection imply the manipulation of genes - even if unwittingly. Brewing, cheese making, baking, etc. all rely on mastering fermentation using a variety of microorganisms, and are as old as human history.

With the advent of recombinant DNA⁠¹, however, the power of biotechnology has increased dramatically, and has sparked the imagination of scientists and laymen alike. Applications of biotechnology are flourishing particularly in the fields of human health and agriculture. Concomitantly, however, growing concerns about potential risks and ethics have been debated amongst scientific and public fora, and have sometimes led to moratoria issued by the scientific community itself or imposed by legislative powers.

2. BIOTECHNOLOGY AND ANIMAL PRODUCTION

The challenge faced by animal production is to provide society with food products that meet their evolving nutritional requirements, within specific economic and environmental constraints. Based on the central tenet “\( P = G + E \)”, namely that the Phenotype of an animal reflects its intrinsic genetic aptitude or Genotype as expressed in a given Environment, animal breeders have adopted a two-pronged approach towards improving the quality of their production. On the one hand, they have learned to master the environmental component by improving animal husbandry and nutrition practices, disease prophylaxis and treatment. On the other hand, artificial selection has allowed to continuously improve the genetic make-up of domestic species. The implementation of sophisticated biometrical methods in breeding schemes has lead to spectacular genetic progress during the second half of this century.

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¹ desoxyribonucleic acid
Biotechnology has been adopted in the arsenal of tools aimed at improving both the *nurture* (*E*) as well as *nature* (*G*) components of the equation. Applications of biotechnology to improve the *environmental* component have recently been reviewed by Robinson and McEvoy (1993) and include:

- genetically engineering forage species, either to increase their productivity, or to improve their nutritional value,
- genetically engineering microorganisms to produce food additives,
- genetically engineering the gut micro flora,
- production of therapeutic or prophylactic compounds - including vaccines - from genetically engineered microorganisms,
- production of hormones or hormone analogues from genetically engineered microorganisms,
- immunomodulation of physiological processes,
- improved DNA-based diagnostic procedures.

The objective of the present report is to focus on the ongoing efforts applying modern biotechnology to the *genetic* term of the equation, i.e. to more efficiently improve the genetic make-up of livestock species.

### 3. BIOTECHNOLOGY FOR THE GENETIC IMPROVEMENT OF LIVESTOCK

#### 3.1. General overview

Animal breeding operates through the selection of genetically superior animals as parents for subsequent generations. So far artificial selection could therefore only be applied to traits which are “naturally” exhibiting *genetic variation* in the selected populations, i.e. traits characterised by some degree of “heritability”. The rate of genetic progress or of response to selection is a function of:

- the *accuracy of selection*, i.e. the precision in the identification of genetically superior animals;
- the *generation interval*, the shorter the generation interval, the faster the genetic progress;
- the *selection intensity*, i.e. the more the future parental individuals deviate from the average breeding value of their contemporaries, the higher the genetic improvement they will cause.

Biotechnology is being applied to enhance genetic progress through these four factors: increase genetic variation (or the molecular substrate of breeding programmes), increase the accuracy of selection, reduce the generation interval and to increase the selection intensity.

Three major topics can be distinguished in the area of biotechnology applied to the genetic improvement of livestock:

- reproductive technologies,
- livestock genomics and marker assisted selection (MAS), and
- livestock transgenics.

The following table summarises the potential impact of these three areas of research on the four components determining the rate of genetic progress.

<table>
<thead>
<tr>
<th></th>
<th>Genetic variation</th>
<th>Selection accuracy</th>
<th>Generation interval</th>
<th>Selection intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproduction</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Genomics &amp; MAS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Transgenics</td>
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#### 3.2. Reproductive biotechnology
A number of methods have or are being developed to increase the reproductive potential of livestock (2, 8, 9, 10). These include:

- **Artificial insemination (AI):** Especially since the development of efficient semen freezing methods, AI has become the most widespread biotechnology applied to livestock and especially cattle production. By allowing for the widespread use of small numbers of elite sires, AI has had a dramatic impact on selection intensity. In addition, AI has allowed for the implementation of the progeny-testing scheme prevalent particularly in dairy cattle production, and which has had a major impact on the improvement of the herd by increasing the accuracy of selection despite the associated increase in generation interval.

- **Multiple ovulation and embryo transfer (MOET):** By increasing the number of offspring that can be obtained from monotypic species in particular, MOET has the potential to increase genetic improvement by enhancing the selection intensity on the female side. In cattle, however - the species in which this technology is again the most widely disseminated - the major impact of MOET might result from the reduction in generation interval vis-à-vis the conventional progeny-testing scheme, if sires are selected based on the performances of their MOET produced full-sisters rather than the performances of their female progeny: the so-called MOET nucleus scheme. Despite associated technical hurdles, MOET has the potential to play an important role in developing countries where the implementation of a large-scale AI based progeny-testing scheme would be difficult to implement.

- **Oocyte harvesting (OPU), in vitro oocyte maturation (IVM), in vitro fertilisation (IVF):** While the number of embryos that can be obtained from a cow/year using MOET is on an average limited to the order of 20 or less, the development of OPU in conjunction with IVM and IVF increases this number by a factor of at least 5. Moreover, OPU can be applied to pregnant animals as well as prepubic animals. The impact of these methodologies on genetic response operates through the same channels as MOET, i.e. increase of selection intensity on the female side and increase of selection accuracy on the male and female side.

- **Nuclear transfer or embryo cloning:** The transfer of totipotent nuclei in enucleated oocytes theoretically allows for the production of large numbers of identical twins or “clones”. The principles underlying embryo cloning are summarised in Figure 1. This opened the prospective to affect genetic response in a variety of ways including selection intensity, selection accuracy and generation interval. Initially, the source of totipotent nuclei were blastomeres. Despite the potential use of first as well as higher order generation blastocysts as nuclei donors, the size of the clones has remained very small. The recent generation of totipotent embryonic stem “ES”-like cells in sheep which will likely be followed by similar developments in other species, might lead to a considerable increase in the efficiency of embryo cloning (3).

- **Sex selection:** Recent improvements in flow cytometric sorting now allows for the effective separation of viable X and Y-bearing sperm. While the numbers of cells recovered are incompatible with conventional AI practices, they are sufficient when combined with IVF techniques. This might become the method of choice to generate embryos of a desired sex. Embryo sexing can also be achieved by micro biopsy and sex determination using polymerase chain reaction (PCR) amplified Y-specific sequences. This approach, however, is economically only justified in very exceptional circumstances.

- **Gamete and embryo cryopreservation:** Most methods described are only effective when used in conjunction with gamete and embryo freezing methods. In addition cryopreservation plays a crucial role in conservation programmes aimed at maintaining genetic diversity.

### 3.3. Livestock genomics and marker assisted selection (MAS)

Advances in molecular genetics, boosted by the 'Human Genome Initiative', now allow for the development of unlimited numbers of genetic markers, the fundamental tool of the geneticist. These markers can be used to locate genes underlying phenotypic traits on the corresponding genome maps using linkage strategies. This mapping is the first step in the process referred to as *positional cloning* which culminates in the isolation of the causal gene and mutation (5).
For the first time, these methods allow the dissection of production traits into their individual Mendelian components or genes. Table 2 lists the monogenic traits that have been mapped in livestock using this approach. For polygenic traits, to which the majority of production traits belong, these genes are referred to as Quantitative Trait Loci (QTL). Recent experiments studying growth and carcass characteristics in pigs, as well as milk production in cattle, have convincingly demonstrated the feasibility of this QTL mapping approach in livestock (1, 6).

Understanding the molecular biology of production traits is of importance in several respects. It is strongly believed that the identification of QTL will allow for the implementation of novel “marker assisted” selection schemes (10). MAS is expected to increase genetic response by affecting all four relevant factors. Mapping genes explaining breed differences for economically important traits will allow their introgression in other populations by marker aided backcrossing, therefore increasing the genetic variation usable as substrate for selection programmes. Probably the most publicised example of this is the search for the genes causing hyperprolificity of Chinese pig breeds. Adding information on mapped QTL on top of their own performance data and that of relatives, will increase accuracy of selection especially by explaining Mendelian sampling variance. As the marker genotype is obtainable at virtually any stage of development and irrespective of sex, there is considerable potential for reduction in generation interval. Finally, marker genotyping will become considerably cheaper than phenotype collection allowing selection for more traits amongst more individuals than previously and therefore increasing the selection differential or intensity.
<table>
<thead>
<tr>
<th>Species</th>
<th>Locus</th>
<th>Trait</th>
<th>Position</th>
<th>Gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>MH</td>
<td>Malignant hypertemia</td>
<td>6</td>
<td>CRC</td>
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<tr>
<td></td>
<td>I</td>
<td>Dominant white coat colour</td>
<td>8</td>
<td>KIT</td>
<td>Johansson et al., 1992; Johansson Moller et al., 1996</td>
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<td></td>
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<td>6</td>
<td>?</td>
<td>Mariani et al., 1996</td>
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<td></td>
<td>Rn</td>
<td>Muscle glycogen content</td>
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<td>?</td>
<td>Milan et al., 1995 &amp; 1996; Mariani et al., 1996</td>
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<td>Guérin et al., 1993; Edfors-Lilja et al., 1995</td>
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<td></td>
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<td>Weaver</td>
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<td>Georges et al., 1993b</td>
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<td></td>
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<td>2</td>
<td>?</td>
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<td></td>
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<tr>
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<td></td>
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<td></td>
<td>SLD</td>
<td>Sex linked dwarfism</td>
<td>Z</td>
<td>GHR</td>
<td>Huang et al., 1993; Duriez et al, 1993</td>
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Moreover, understanding the molecular biology underneath complex traits might reveal novel mechanisms of gene action requiring adjusted selection schemes for their optimal exploitation. A good illustration of this point is the discovery of the non-Mendelian segregation mode characterising the callipyge muscular hypertrophy in sheep, and referred to as dominant negative imprinting (4). Molecular understanding of this entity has shown the inadequacy of conventional selection programmes for its exploitation and has suggested matings between specific but phenotypically normal paternal and maternal lines to yield 100% callipyge offspring.

Finally, genetic markers allow quantitation of the genetic diversity existing between domestic livestock populations, which is essential for the implementation of cost-effective conservation strategies (7).

3.4. Transgenics

While conventional breeding strategies as well as MAS are restricted to the exploitation of genetic variation preexisting within the species if not breed of interest, transgenics has opened the exciting possibility to exploit variants across species barriers or even create de novo.

Two major approaches for the production of transgenic animals have to be distinguished: 1) by microinjection of DNA constructs in the male pronucleus of one-cell stage embryos (Figure 2), and 2) by genetic targeting of totipotent (f.i. “ES”) cells in culture, followed by either nuclear transfer in enucleated oocytes or microinjection into blastocysts (Figure 3). While in the former procedure, the transgene is integrated randomly into the genome (which may affect its expression pattern), gene targeting mediated by homologous recombination is locus-specific.

Until very recently, the first approach was the only option available for livestock species in the absence of suitable totipotent livestock cell lines. The recent description of ovine “ES” cells and their use to produce chimeric sheep, however, demonstrates that gene targeting methods might become available for animal production in the near future as well (3).

A variety of transgenic projects are being conducted (9) with the aim to 1) enhance growth and improve carcass characteristics, 2) increase milk production and alter milk composition, 3) increase disease resistance, and 4) improve wool production. However, the application of transgenics applied to livestock species that has proven most successful so far is in the area of gene-pharming or the use of livestock species as expression systems for the production of high value protein products.

3.5. Survey of ongoing work

In order to evaluate the activities in the field of biotechnology applied to the genetic improvement of livestock, a questionnaire-based survey was organised among the 143 OIE Member Countries, of which 46 responded.

The following general conclusions can be made from this survey:

- Reproductive biotechnology: This is by far the area of biotechnology applied to animal production that experiences the most widespread dissemination. Most if not all responding Member Countries are investing in research aimed at improving AI and ET efficiency, and are often active into the more sophisticated embryo manipulation techniques. AI and ET have made it to the field in most countries, although to varying extents.

- Genomics and MAS: Despite its recent development, research into genomics applied to animal production is remarkably widespread, even if still absent from the research programmes of a number of economically less developed countries. A large number of programmes are underway to identify genes underlying a variety of production traits and this in most domestic species. Encouraging results are already being reported which are likely only the beginning of a wealth of data to be generated in the near future.

At present DNA-based genetic markers are primarily used for identification and parentage verification, as well as for the control of single-gene disorders. Widespread diagnosis for bovine leucocyte adhesion disease in cattle and porcine stress syndrome in pigs are only two illustrations of the emergence of this field.

Figure 2
Whether MAS will have a major impact on the selection for polygenic traits depends on the proportion of the genetic variance that can be explained with mapped genes, as well as on the development of cost-effective selection schemes incorporating marker data. The fact that a lot of research is being conducted in the area of MAS selection increases the likelihood of a successful outcome.

- **Transgenics:** As previously mentioned, the most commonly cited application of transgenic techniques is in the area of gene-pharming, driven by funding from the private sector. Research efforts targeting the use of transgenics to enhance conventional livestock production do exist but in limited numbers and confined to large research centres. This is likely due in part to the high costs of these efforts, the difficulties to predict the outcome of the experiments, and the negative public perception (at least in some countries) associated with the method. The recent development of totipotent cell lines in sheep will likely create a resurgence of research activities in this field.
Figure 3

ES cells

Selectable markers

Gene Y

A
B

Homologous recombination

Positive (+B) / Negative (-A) Selection

Microinjection in blastocyst

Transfer into foster mother

Somatic chimera

Germline chimera

DNA analysis
4. FUTURE CHALLENGES

Increasing production efficiency for animal products, either by manipulation of the environment or of the genotype, remains a major component of the available means to achieve proper feeding of the growing world population in a sustainable way. Biotechnology is an essential part of the arsenal of tools needed to reach this objective. As the methods continue to develop, the contribution of biotechnology is likely to increase in the future.

The major problems related to the application of biotechnology in animal production as identified by the responding Member Countries were primarily of three types:

1. a growing distrust in and fear of biotechnology by the general public. These concerns were typically voiced by North American and Western-European countries.

2. a scarcity of properly educated scientists and technicians and a lack of support for projects aimed at applying biotechnology to livestock production. These concerns were primarily expressed by countries other than North American and Western-European.

3. doubts about the cost-effectiveness of biotechnology applied to animal production.

Based on these identified problems facing biotechnology, the following supportive actions were suggested by the OIE Member Countries:

1. Organisation of campaigns to inform the general public about the nature of biotechnology.

2. Assistance with education in biotechnology of the future working force by fostering conferences and exchange programmes between countries.

REFERENCES


