

IMPACT OF BIOTECHNOLOGY ON ANIMAL BREEDING AND GENETIC PROGRESS

GENGLER N. AND DRUET T.

*National Fund for Scientific Research, B-1000 Brussels, Belgium
Animal Science Unit, Gembloux Agricultural University,
B-5030 Gembloux, Belgium.*

Abstract

Animal breeding is a field related to a whole range of biotechnologies. The impact of a biotechnology can be measured by the influence it has on genetic progress. According to the type of biotechnology considered, different component of genetic progress may be affected: accuracy of prediction, generation interval, intensity of selection and genetic variance. The first type of biotechnologies affects the efficiency of male and female reproduction: artificial insemination, multiple ovulation, in-vitro-fertilization, ova pick-up, embryo-transfer, twinning, sexing of semen and embryos cloning and selfing. The impact of these technologies is mainly in the enhanced distribution of superior germplasm and the selection intensity, but also in the accuracy obtained when testing animals. In the past, artificial insemination has been a very successful biotechnology, enhancing greatly the genetic progress. A secondary, negative, impact is that these biotechnologies affect indirectly genetic diversity and therefore reduce genetic variance. A second group of biotechnologies can improve determination of the genetic merit of animals. These are all the techniques relate to quantitative or economical trait loci (QTL/ETL), their detection and use. Their main feature is the early availability in life, therefore allowing an earlier and more accurate selection. Two direction of research exists: detection of markers for the unknown QTL and direct use of a potential candidate genes as QTL/ETL. QTL/ETL will have a major impact on animal breeding especially if their use in future breeding programs can be optimized. A last type of biotechnologies with a large potential to affect animal breeding in the future are those with the ability to transform artificially DNA. The impact of these technologies is however still no very clear especially as gene expression and other issues remain unsolved. Biotechnology had, has and will have a major impact on animal breeding and genetic progress. To a certain extend animal breeding is a very promising field to use biotechnology as the past has already proven.

1. Introduction

Precise definition of biotechnology is an arduous and difficult task. Therefore not only one definition, but a whole range exists from very general to very restricted ones. One broad definition could be to define biotechnologies as a group of technologies that are based on the use and mostly on the transformation of living organisms to provide services and products. Animal breeding on its own is therefore by this definition a biotechnology because its ultimate goal is the transformation of animal germplasm in order to create for animal production new generations of animals that are superior to current ones.

The definition of superior is here rather large as indeed focus of animal breeding has been shifting in recent years rapidly from only short-term production goals to functionality of animals, reduction of production costs, consumer perception and quality of products and therefore to overall sustainability and long-term economic return of animal production.

Classical animal breeding has two major characteristics. First, it uses the existing genetic variability, it does not create it artificially. Introduction of new alleles or characteristics is done by cross-breeding of different populations and/or selection of animals in existing populations. Second, detection of superior animals is done using phenotype and advanced statistical methods (BLUP) allowing the separation of genetic and environmental effects.

2. Biotechnologies and Animal Breeding

Widespread use of some biotechnologies in animal husbandry resulted (Van Vleck, 1981) and will result in a major impact on genetic progress. Different types of biotechnologies may have influence on both animal breeding and the resulting genetic progress through three main ways:

- Biotechnologies can affect efficiency of reproduction and therefore also selection programs: artificial insemination, embryo transfer, sexing, cloning and other related techniques (e.g., Ruane and Thompson, 1991; van Vleck, 1981).
- Biotechnologies can improve determination of genetic values of animal: genetic markers, candidate genes and other related techniques (e.g., Georges *et al.*, 1995; Renaville *et al.*, 1997).
- Biotechnologies can transform artificially the genome at the DNA level: genetic engineering, gene transfer and related techniques (e.g., Solter, 1981).

The objective of this study was to review the importance of the most common current and likely future biotechnologies on animal breeding and genetic progress.

3. Some Basics of Animal Breeding and Related Issues

3.1. GENETIC GAIN EXPRESSED AS SUPERIORITY OF SELECTED GROUPS

In order to be able to consider what effect biotechnologies may have, it is important to formalize equations that describe genetic gain ΔG (Falconer, 1989):

$$\Delta G = r_{G\hat{G}} i \sigma_G \quad (1)$$

where $r_{G\hat{G}}$ is the correlation between the actual and predicted additive genetic value, also called accuracy of evaluation, i is the selection intensity (expressed as the standard normal selection differential) and σ_G is the genetic standard deviation. Biotechnologies will act mainly by allowing an increase in selection intensity (by enhancing reproductive efficiency of animals) and by increasing the accuracy of evaluation (by additional information or increase in number of progenies). However, intensive use of some biotechnologies could also affect genetic variance (e.g., increasing the inbreeding trend or reducing genetic diversity).

The equation (1) can be modified to reflect genetic progress by time period (year) $\Delta g = \Delta G/\text{yr}$:

$$\Delta g = \Delta G/\text{yr} \frac{\Delta G}{1} = \frac{r_{G\hat{G}} i \sigma_G}{1} \quad (2)$$

where 1 is the generation interval in years. In reducing generation interval, some biotechnologies could then increase genetic progress by time period.

Formula (2) is only theoretical because based on the hypothesis of equal accuracies, selection intensities and generation intervals in different selection paths. In reality this hypothesis is obviously not true, especially if one considers males and females. There are four different selection pathways (van Tassel and van Vleck, 1991): sire of sire (SS), sire of dam (SD), dam of sire (DS), dam of dam (DD) and formula (2) can be developed (Rendel and Robertson, 1950):

$$\Delta g = \frac{\Delta G_{SS} + \Delta G_{SD} + \Delta G_{DS} + \Delta G_{DD}}{1_{SS} + 1_{SD} + 1_{DS} + 1_{DD}} \quad (3)$$

3.2. PROGENY DIFFERENCES

The equations given above reflect long-term and population wide genetic gain. Breeders are also interested in genetic gain in a short-term perspective. To express short-term gain, progeny superiority formula (1) can be modified to be (van Vleck, 1981):

$$\Delta G_p = \frac{\Delta G_S + \Delta G_D}{2} = \frac{r_{G_S G_S} i_S \sigma_G + r_{G_D G_D} i_D \sigma_G}{2} \quad (4)$$

Equation (4) reflects population wide expected progeny difference. For a given animal i the expected additive genetic value would be (Mrode, 1996):

$$\hat{a}_i = \frac{\hat{a}_{i_S} + \hat{a}_{i_D}}{2} \quad (5)$$

In most situations equation (5) will also be close to the expected phenotypic progeny difference. Often studies on impact of biotechnologies are limited to genetic additive aspects. However, the expected progeny difference will be affected by inbreeding and non-additive genetic effects (e.g., dominance effects). Both these parameters can heavily be influenced by biotechnologies (van Raden and Hoeschele, 1991). A more general formula (6) assessing the total superiority of a given animal i (Δp_i) without own records should include both these effects:

$$\Delta p_i = \frac{\hat{a}_{i_S} + \hat{a}_{i_D}}{2} + \hat{d}_{i_S, i_D} + \hat{\alpha}_F F_i \quad (6)$$

where \hat{d}_{i_S, i_D} is the parental dominance effect [9], $\hat{\alpha}_F$ is the inbreeding depression per percent inbreeding and F_i is the inbreeding coefficient in percent. This formula could be extended especially towards molecular information as we will see later. Other environmental, genetic, and/or genetic x environmental effects could be introduced (van Raden and Hoeschele, 1991). In most polygenic cases the three terms in (6) will be enough, other genetic terms as additive x additive or additive x dominance effects are considered to be small (Misztal *et al.*, 1998) or can not be correctly estimated and environmental or genetic x environmental effects are considered to be negligible in most current animal breeding situations.

The concepts of parental dominance and inbreeding need some explanations. Parental dominance (Hoeschele and van Raden, 1991) effects reflect the interaction between alleles coming from sires and dams at a given loci. Intensive use of biotechnologies can create larger sire x dam families and therefore increase eventual necessity to take these effects into account. Inbreeding of an animal i reflects percentage of homozygotic pairs of alleles and is computed as the probability that the two alleles at a given loci are descendent from one allele of a common ancestor of sire and dam (Weigel and Lin, 2000). Inbreeding is also an important issue because a lot of biotechnologies will result in fewer active breeding animals or even new types of animals that have higher or even extreme inbreeding coefficient.

4. Biotechnologies Affecting Mainly Efficiency of Reproduction

Genetic improvement is highly dependent on efficiency of reproduction in order to disseminate superior germplasm. Early biotechnologies focused very strongly on the improvement of this biological function. Indeed, a lot of economically important animal species had very limited reproduction capacity.

4.1. MALES: ARTIFICIAL INSEMINATION

Artificial insemination (AI) can be considered as the first large scale reproduction biotechnology. Since its earliest commercial days in the 1930's AI has become an extremely common way to breed females at least in bovine, porcine and similar species (Foote, 1981). Most of its development occurred in dairy cattle and dairy production, therefore we focus on the impact of AI in this animal production. If its first use was often more to avoid diseases, its real impact, as appears nowadays, was on genetic progress (Foote, 1981).

AI acts on genetic progress in several ways. First AI allows to heavily increase the selection intensity (van Vleck, 1981). The number of required bulls to breed the available cows is heavily reduced. At the same time, the widespread use of tested older and the progeny testing of younger bulls allow to achieve very accurate estimations of breeding values. In dairy cattle, frozen semen of AI bulls can also be easily shipped, therefore it disseminates the favorable alleles in a wider population across countries but also world-wide. A surprising side effect of this seems to be that despite heavy selection genetic variances are not yet decreasing in most species undergoing heavy selection due to AI. A direct consequence of all these facts was a strong increase of the genetic progress as described by formula (1) and in dairy cattle (van Vleck, 1981).

However, some undesired side effects can appear. The heavy use of the best males resulted in a strong increase in inbreeding and a loss of genetic diversity. For the moment the annual increase in the inbreeding trend in the USA in Holsteins dairy cows is estimated to be near to 0.5 % (Wiggans *et al.*, 2000)! Also examples, like the Holstein bull Skalsumer Sunny Boy being used at least for over 1,000,000 first inseminations, show the risks of rapidly shrinking breeding populations. Recent research in France (Maigel *et al.*, 1996) showed that the local Holstein population with over 5 million animals was in reality behaving as if this population consisted of less than 100 unrelated animals!

4.2. FEMALES: MULTIPLE OVULATION, IN-VITRO FERTILIZATION, OVA PICK-UP, EMBRYO-TRANSFER AND TWINING

Multiple ovulation (MO) and embryo transfer (ET, often called together MOET) is a procedure that is similar to AI, but affects the reproduction ability of females (Seidel and Seidel, 1981). MOET is therefore a biotechnology that makes species like cattle multiparous, allowing the best cows to have more than the natural number of descendants. Its most important feature is the increase of selection intensity in the female selection paths (Ruane and Thompson, 1991), especially the selection of bull

dams less but better dams therefore improving the bull dam pathway as shown in formula (2). Unfortunately good females, especially in dairy cattle, are often subject to preferential treatment to let them appear even better and it was and is always quite difficult to identify a truly superior female animal (van Vleck, 1999).

By application of MOET techniques, the number of full-sibs families increases (van Raden *et al.*, 1992). Therefore, it is more important to include dominance effects in order to get more precise breeding values (van Raden *et al.*, 1992). The gain of accuracy of breeding values through inclusion of dominance effects is quite important because animals produced by MOET techniques are often used as top-reproducers.

The splitting of embryos is a way to artificially produce twins. It is a rather old technique that was used as early as during the late 1980's to create dairy AI bulls (Jackbuilt Great Divide-ETS and Duplicate-ETS) (ABSglobal). This technique which is nowadays quite often used for superior females increases the number of potential descendants per cow. Unfortunately survivability of embryos is affected.

Two other modern enhancements to MOET are in-vitro fertilization (IVF) and ova pick-up (OPU). IVF does not play in cattle for example the role it has in human reproductive biotechnologies. But it can help produce embryos from some females that do not react properly to MO, or that are in general poor health. Its implications for genetic improvement are low. OPU however can greatly reduce generation interval as it provides a method to use very young animals in selection schemes.

An interesting way to use MOET and related techniques is in nucleus breeding schemes. In such schemes parts of the whole population are subject to better performance recording eliminating preferential treatment and more intense (e.g., MOET) and earlier (e.g., OPU) selection.

Despite a lot of very positive simulations, closed and therefore totally disconnected from the whole population, closed nucleus herds were not successful in cattle breeding. In swine and poultry most breeding companies run apparently closed nucleus schemes. But one might suspect that these schemes are in reality often not totally closed, but subject to the introduction of external superior animals. Totally open nuclei which are basically combinations of future bull dam station testing and MOET technologies seem to be a very promising way to enhance genetic progress in cattle because they combine optimal or at least better bull dam selection with classical progeny testing of the produced sires.

Finally, a very special type of biotechnology affecting female reproduction is twinning. Especially in beef cattle were obtaining more than one calf per calving can greatly enhance production efficiency this technology can be useful. At the same time twinning and ovulation rate, a rather closely related trait, can be both measured and are partially genetic. Therefore the Meat Animal Research Center in Clay Center, Nebraska has initiated a selection experience in this field (van Vleck and Gregory, 1996). They were able to increase the rate of twins from around 3% to over 20% in less than 20 years (van Vleck and Gregory, 1996).

4.3. SEXING

Sexing of semen and of embryos provides in species and production circumstances where one sex is preferred, a way to produce the wanted type of animal (Betteridge *et al.*, 1981; Johnson *et al.*, 1998). Sexing of embryos is a technique that is rather reliable nowadays and currently used on a rather large extent. Some progress was recently made in the field of sexed semen. One problem is the price of sexing and the eventually reduced fertility of this semen. The other issue is that sexing has to be economic what means that the extra-price to be paid for sexed semen has to be in relation with the extra income.

4.4. CLONING AND SELFING

An apparently new technique that is widely covered in the media is cloning. In fact nucleus transfer is not really a new technique. The real new point is that it allows now to clone an adult mammal (Campbell *et al.*, 1996). This is indeed a major enhancement compared to the original techniques that required embryonic nucleus (McKinnell, 1981).

From the animal breeding point, cloning can have multiple aspects and the assessment of its economical value is still rather uncertain (Dematawewa and Berger, 1998). First it is a possibility to multiply phenotypically outstanding individuals. Unfortunately, even performances done by the same animal show a correlation, often called repeatability, of less than 1 (van Vleck, 1999). Therefore multiplying a phenotypically outstanding individual is by no means a guaranty that the resulting clones will be outstanding. Again phenotypically outstanding animals are also very sensitive to preferential treatment. Similarly, only animals with precisely estimated genetic merits could be interesting candidates for cloning, this means especially sires and here doubts exist why we should clone an outstanding sire that can reproduce easily through AI? Use of cloning should require precise evaluation of each clone family, therefore a lot of clone-testing with resulting costs. An advantage of cloning, is that we can integrate dominance effects into models (every clone of the same family has the same dominance effect) and reproduce them.

However, reproduction through clones would heavily increase selection intensity in the first generation, or better limit the population used for reproduction, but afterwards would result in no or severely reduced genetic progress as we duplicate always the same animals leading to the more fundamental following problem. Cloning is by definition conservation of existing germplasm. This means that, except for mutations and aging of DNA, at least nuclear DNA will stay the same. But genetic improvement is based on the creation of new, eventually favorable, combinations of parental germplasm at every mating. Dairy cattle selection shows that this is a highly efficient way to work. It is usually, in large populations such as Holsteins, easy to find sons that easily surpass their sires. Another more theoretical argument against cloning in general animal improvement is that extensive use of clones, as in plants, reduces genetic variation and therefore long-term progress that can be achieved. But cloning has truly high potential to multiply new genotypes obtained by natural and artificial means.

From all biotechnologies, cloning is certainly the one mostly reducing diversity. We already discussed above the impact of such a reduction on selection but reduction of diversity can also have other dangerous consequences. First, heavily selected animals produce optimally only in specific conditions. If environmental (including new techniques) conditions change, then those animals would show poor adaptability to new conditions and their would be no way back. This means that cloning forbids any change in selection path because animals are fixed, not adaptable to new needs, new economical conditions, they will not resist to new disease.

Selfing is based on the fusion of two X gametes from a bull and the creation of what could be called selfs (van Vleck, 1981). This would create animals that are potentially highly expressive of the parental genotype. Some technical issues are obviously not yet solved. But for testing of animals that are not expressing phenotypes, as dairy bulls, this can be a clear alternative. Unfortunately such animals are 50% inbred, therefore we have here a major problem because this means parents need to be totally free of any lethal or sublethal alleles. Current theory to account for inbreeding but also dominance effects under inbreeding in such extreme animals might not work.

5. Biotechnologies Improving Determination of Genetic Values of Animals

Estimation of breeding values is for the moment essentially done by analysis of the phenotype or the phenotypic performances of the animals. Advanced statistical methods (BLUP) are used to separate genetic and environmental effects, relating also animals to each other according to their genetic relationships (Henderson, 1984). Unfortunately the phenotype can not always be recorded due to physiological (e.g., bulls give no milk, boars give no piglets, dairy cows calf only around 2 years of age) or other reasons (e.g., recording expensive as for beef quality traits), or the recording of the phenotype is inaccurate or imprecise (e.g., calving ease). Since several years a large progress has been made in the understanding and description of DNA and the actions of certain genes. Under the influence of this research animal breeders started to investigate if variations in DNA (polymorphism) of some animals can be linked to differences in production or other economically important traits (e.g., milk production). But there are several basic problems inherent to whole genome. First, one has to determine the functional structure of the chromatin. Then inside this structure functional, often called genes, and unfunctional regions can be spotted.

Main advantages of QTL/ETL in breeding programs are: 1) an increase in accuracy in selection through additional information directly related to the genotype; 2) a possibility to reduce generation interval by adding a new selection stage at earlier age because QTL/ETL allow to make observations that are not sex or age dependent. QTL/ETL could also increase the efficiency of introgression or be used in the prediction of heterosis by genetic distance.

The detection, the use of QTL/ETL and their potential role in animal genetics and breeding will be emphasized.

5.1. DETECTION OF QUANTITATIVE OR ECONOMICAL TRAIT LOCI

This step basically consists in the study of potential links between DNA polymorphisms and production or similar traits. The idea is that a specific gene called hereafter QTL/ETL (quantitative or economical trait loci) is responsible for part of the phenotype. Two different approaches are currently used to detect QTL/ETL. If a known gene is used as candidate to be the QTL/ETL the method is called candidate gene approach. If the DNA polymorphism is only considered being the marker for a DNA region containing the QTL/ETL the method is called genetic marker approach (eg, Georges *et al.*, 1995).

The candidate gene method (e.g., Renaville *et al.*, 1997) is rather dependent on a priori knowledge on a genetic and physiological level. First, genes and their DNA sequences need to be known. Then selection of the right gene should be based on good knowledge of its position inside a metabolic pathway. Additive effects for the different alleles for these candidate genes are then compared to each other in order to establish allele substitution effects. This can be done using different techniques and on a population or family level.

Genetic markers are also based on known DNA polymorphism. But contrary to candidate genes these polymorphisms are mostly not functional DNA regions, but nonfunctional. The markers used are mostly microsatellites, regions that are highly polymorphic. Through the observation of transmission of markers over generations, transmission of marked DNA parts can be traced through pedigree and linked to phenotypic differences. This QTL/ETL detection method is basically family oriented.

5.2. USE OF QUANTITATIVE OR ECONOMICAL TRAIT LOCI

Detection of QTL/ETL is one point, but the main issue is their integration in current breeding programs. Larzul *et al.* (1997) gave some details on the potential impact of its use. Also, different technical problems are linked to QTL/ETL use.

First detection of QTL/ETL is done in families or sub-populations, therefore their use in the whole population has to be done carefully or restricted to known families where their segregation is known.

QTL/ETL detection is done on a single trait basis, therefore only for certain traits, seldom for groups of traits. Therefore pleiotropic effects on other traits can not be excluded and have to be studied carefully. A real-life example is the muscular hypertrophy gene. This gene produces not only double muscled animals but seems to have a pleiotropic effect on several malformations.

Also current research is focusing on additive QTL/ETL effects. But, the remaining polymorphism at QTL/ETL levels needs to be explained. One possible answer is that there might be favorable dominance effects that keep different alleles in the population despite heavy selection.

A last important issue is that in a lot of situations molecular information is only available for some animals.

QTL/ETL could be used in many ways. Different methods could differ by the use or not of a priori levels of QTL/ETL effects, or by the joint estimation of polygenic and

QTL/ETL effects. Obviously the second way to proceed would be ideal. Some obvious uses of QTL/ETL could be as follows:

First, a priori knowledge of the level of QTL/ETL effects in families/sub populations/populations could be used for animals without any other information than their parent average (formula (5)) extending formula (6). This is typically the use most people think about for the moment. This could for example be used to identify earlier superior AI test sires. A second use of QTL/ETL is typically inside nucleus breeding schemes where also joint evaluations might become possible. In the near future joint evaluations including additive and dominance QTL/ETL effects might become feasible even in rather large populations. Then the predicted phenotype would be:

$$\Delta p_i = \frac{\hat{a}_{iS} + \hat{a}_{iD}}{2} + \hat{d}_{iS, iD} + \hat{\alpha}_{FFi} + \sum \hat{q}_{ai} + \sum \hat{q}_{di} \quad (7)$$

All polygenic effects are reduced for the QTL effects of the animal i where $\sum \hat{q}_{ai}$ represents the sum of the additive and $\sum \hat{q}_{di}$ the sum of the dominance QTL effects.

Such developments will then be one element in the development of new mating schemes emphasizing optimal use of all information, and improved data exchange through new information technologies as INTERNET (Misztal *et al.*, 1998). Figure 1 modified from Misztal *et al.* (1998) gives an overview how such a scheme could be structured.

6. Biotechnologies Transforming Artificially the Genome at the DNA Level

Until this point all presented biotechnologies were based on naturally existing DNA and tried to evaluate, use, disseminate and adapt it optimally, but without intervening artificially on it. Genetic engineering however exists and can be used in superior animals and plants. Here we enter a rather difficult field and the real impact of these technologies on animal breeding may be not yet known. In fact genetic engineering in domestic species is a way to suppress species frontiers that limited introgression of certain genes (alleles) into populations. For example until now only crossbreeding stress negative Large White sows with stress positive Pietrain boars and backcrossing towards Pietrain but retaining the stress-negative alleles in the population allowed to obtain stress negative Pietrain pigs (Leroy and Verleyen, 1998). In the future desired genes or alleles can be directly introduced. For example then some dairy cows may be starting to produce high value protein or have no lactose. Such special animals could then be multiplied through cloning. Direct introduction of QTL/ETL in population will be, but not quickly a widespread technique. Indeed the real major issues with genetic engineering is gene expression and simple gene transfer is not enough. In fact current experience with domestic or other higher animals seem to show that expression of introduced genes is a major problem.

Impact of biotechnology on animal breeding and genetic progress

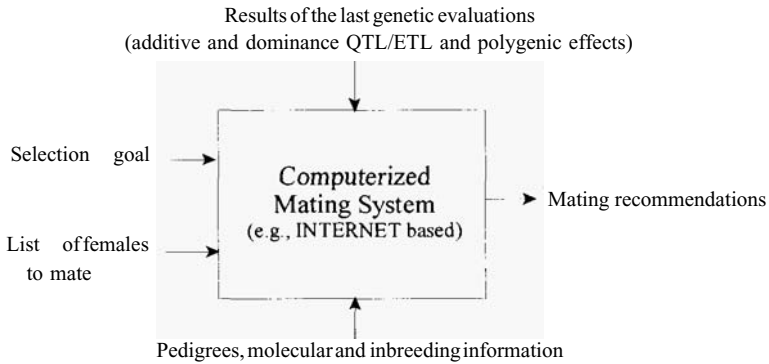


Figure 1. Computerized mating system showing the potential use of QTL/ETL.

7. Conclusions and Implications

There is a natural link between biotechnologies and animal improvement which is in itself a biotechnology. This link was therefore very important and animal breeding and genetic progress have been greatly enhanced by the use of reproductive biotechnologies as artificial insemination and embryo transfer. These old technologies are under continuous development leading to sexing of semen and embryos and cloning. Their influences are especially in the improved selection intensities and the more rapid dissemination of superior germplasm. But they have some negative side effects especially through increasing inbreeding and decreasing genetic diversity.

Currently, much research is focusing on the detection of quantitative or economical trait loci. The ultimate goal of this is to improve accuracy of detection of genetic merit. Despite large research efforts these techniques will probably never explain all the genetic variation. Therefore they need to be integrated in polygenic evaluations. Also the real issue in the future will be the evaluation and introduction of molecular information into genetic evaluation and their use in selection schemes that will be taking advantage of advanced computerized mating schemes.

Direct and artificial modification of the germplasm of domestic animal is the current ultimate type of biotechnology. These genetic engineering techniques provide ways to adapt genetic material to specific needs but some technical, but also ethical issues are not yet solved.

Biotechnology had, has and will have a major impact on animal breeding and genetic progress. To a certain extent animal breeding is a very promising field to use biotechnology as the past has already proven.

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