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REVIEW ARTICLE

Genetic engineering applications in animal breeding

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Abbreviations: ES: embryonic stem cells
ESR: estrogen receptor locus
IGF-I: insulin-like growth factor I
MAS: Marker-assisted selection
QTL: quantitative trait loci

This paper discusses the use of genetic engineering applications in animal breeding, including a description of the methods, their potential and current uses and ethical issues. Genetic engineering is the name of a group of techniques used to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms. Important applications of genetic engineering in animal breeding are: 1) Marker-assisted selection (MAS). The objective of this technology is to increase disease resistance, productivity and product quality in economically important animals by adding information of DNA markers to phenotypes and genealogies for selection decisions. 2) Transgenesis, the direct transfer of specific genes/alleles between individuals, species, or even Kingdoms, in order to change their phenotypic expression in the recipients. Compared to the 'traditional' improvement techniques based on phenotypic information only, these *gene-by-gene* techniques allow theoretically a more complete management of animal genomes for animal breeding. In spite of high expectations and new technical developments, its actual efficiency is not always high, as they require a thorough knowledge of functional genomics, and pose additional technical, economical and ethical problems. The possible role for cloning adult animals in breeding is also discussed.

Genetic engineering is the name of a group of techniques used for direct genetic modification of organisms or population of organisms using recombination of DNA. These procedures are of use to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Izquierdo, 2001; Karp, 2002). Most techniques are related to the direct manipulation of DNA oriented to the expression of particular genes. In a broader sense, genetic engineering involves the incorporation of DNA markers for selection (marker-assisted selection, MAS), to increase the efficiency of the so called 'traditional' methods of breeding based on phenotypic information. The most accepted purpose of genetic engineering is focused on the direct manipulation of DNA sequences These techniques involve the capacity to isolate, cut and transfer specific DNA pieces, corresponding to specific genes (Lewin, 1999; Klug and Cummings, 2002).

The mammalian genome has a larger size and has a more complex organization than in viruses, bacteria and plants. Consequently, genetic modification of animals, using molecular genetics and recombinant DNA technology is more difficult and costly than in simpler organisms. In mammals, techniques for reproductive manipulation of gametes and embryos such as obtaining of a complete new organism from adult differentiated cells (cloning), and procedures for artificial reproduction such as in vitro fertilization, embryo transfer and artificial insemination, are frequently an important part of these processes (Murray et al. 1999; Izquierdo, 2001).

Current research in genetic engineering of animals is oriented toward a variety of possible medical, pharmaceutical and agricultural applications. Also, there is an interest to increase basic knowledge about mammalian genetics and physiology, including complex traits controlled by many genes such as many human and animal diseases (Houdebine, 1998; Lynch and Walsh, 1998; Montaldo and Meza-Herrera, 1998; Schimenti, 1998; Eggen, 2003). The interest in genetic engineering of mammalian cells is based in the idea of, for example, use gene therapy to cure genetic diseases such as cystic fibrosis by replacing the damaged copies of the gene by normal ones in foetuses or infants (gene therapy) (Izquierdo, 2001;

NHGRI, 2001; Coutelle and Rodeck, 2002). Genetically engineered animals such as the 'knockout mouse', in which one specific gene is 'turned off', are used to model genetic diseases in humans and to discover the function of specific sites of the genome (Majzoub and Muglia, 1996). Genetically modified animals such as pigs will probably be used to produce organs for transplant to humans (xenotransplantation) (Murray et al. 1999; Prather et al. 2003). Other applications include production of specific therapeutic human proteins such as insulin in the mammary gland of genetically modified milking animals like goats (transgenic animals, bioreactors) (Murray et al. 1999; Wall, 1999). These techniques may be used to increase disease resistance and productivity in agriculturally important animals by increasing the frequency of the desired alleles in the populations used in food production. This can be accomplished by transferring alleles or allele combinations, over expressing or eliminating the expression of particular genes (use of genetic engineering in animal breeding) (Woolliams and Wilmut, 1989; Cameron et al. 1994; Kinghorn, 1998; Fries and Ruvinsky, 1999; Smidt and Niemann, 1999; Hill, 2000; Karatzas, 2003; Felmer, 2004). In addition, these techniques open the possibility of using artificially modified genes to increase the biological efficiency of proteins (Kinghorn, 2003).

The objective of this paper is to review some advances on genetic engineering applications in animal breeding, including a description of the methods, some applications and ethical issues. Here I made emphasis in both the search and use of genomic information for selecting animals and to transfer and use their genes in commercial populations via marker-assisted selection (MAS) or transgenesis.

This review focuses mainly in the methodology to apply genetic engineering directly to animals for genetic improvement.

Several important biotechnological applications such as the production of recombinant proteins in bioreactors (Houdebine, 2002), disease diagnostic (McKeever and Rege, 1999), feedstuff processing (Bonneau and Laarveld, 1999) and production of vaccines (Eloit, 1998), proteins, stem cells, tissues and monoclonal antibodies for use in therapeutics are not included here. The impact of reproductive technologies on animal breeding, not directly related with gene transfer, are reviewed elsewhere (Van Vleck, 1981; Visscher et al. 2000). The possible role for cloning adult animals in breeding is also discussed

USE OF GENOMIC INFORMATION IN ANIMAL IMPROVEMENT

The use of genomic information (sequences or DNA marker polymorphisms) for the genetic improvement and selection of animals requires the knowledge of the effect of physically mapped genes with effects on economically important traits or quantitative trait loci (QTL). This information is also required in order to effectively use transgenesis and MAS for genetic improvement (Lynch and Walsh, 1998; Montaldo and Meza-Herrera, 1998; Van Marle-Koster and Nel, 2003). In MAS, the genomic information is combined with the classical performance records and genealogical information to increase selection accuracy, performing selection earlier in life and reducing costs (Boichard et al. 1998; Elsen, 2003). The traits on which the application of marker-assisted selection can be more effective, are those that are expressed late in the life of the animal, have low heritability, are sex-limited, are expensive to measure or are controlled by a few genes. Examples are longevity, carcass traits in meat producing animals, and diseases or defects of simple inheritance. Expected increments in selection response from MAS for a single complex trait, using known QTL genotypes plus linear model predictions (BLUP), compared to selection on BLUP alone, ranges from -0.7 to 64 percent. In practice, results will depend on many parameters which are likely to be very different for each trait combination and population (Montaldo and Meza-Herrera, 1998; Dekkers and Hospital, 2002). The statistical properties of genetic evaluations (predictions) of animals for quantitative traits obtained through mixed model methodology using phenotypic records and genealogical information as inputs are known as BLUP. Best -means minimum variance of prediction, Linear -because predictions are linear functions of observations, Unbiased -means that the expected value of predictors obtained with linear model have an expected value equal to the expected value of the mean of the breeding values, conditional to data, and Prediction -because involves prediction of random breeding values).

Most experiments on QTL detection in animals allow only the estimation of wide chromosomal regions (practical maximum resolution is of about 1 cM, but usual resolution is about 30 cM) that harbour a QTL in a 'statistical sense', estimated from the effects of some marker haplotypes on quantitative traits (de Koning et al. 2003). Thus, further confirmation is required in order to assure the use of the causative gene. Identification of the causative gene has proven to be difficult. The process to identify the gene responsible for the effect is known either as 'fine mapping' studies (targeting mapping smaller genomic regions) or 'candidate gene' studies (targeting individual genes based on their probable function) (Lynch and Walsh, 1998). In practice, MAS is useful to select genes with effects well identified and precisely located in the genome such as those controlling monogenic recessive diseases such as the pig stress syndrome gene. However, for most recessive alleles with lethal or semi-lethal effects, natural selection will maintain their frequencies very low (Hartl and Clark, 1997) making MAS unnecessary. Unless the additive and non-additive effects for most genes involved in the phenotypic expression of complex, economically important traits are determined, MAS should be regarded just as a tool to increase the rates of genetic gains and not a method to fully open the 'black box' of the genetic control of complex traits, that would render phenotypic selection 'obsolete'. Therefore, the perspectives on the optimum use of DNA

marker information in the framework of a genetic program is still a matter of debate. Quantitative trait loci experiments using crosses between breeds or lines with extreme genotypes for a trait, increases the power of detecting QTLs for that trait, compared to within-family designs. These across population's polymorphisms are not necessarily useful to perform MAS for within-population selection. The favourable allele could be fixed in parental populations and crosses may be commercially irrelevant. Wide genome scans for positioning a QTL using crosses or within-family experiments, are only the initial phase of the search for a true mayor gene involved in a complex trait (de Koning et al. 2003). Another source of complexity for detection and use of QTL for selection is genetic heterogeneity, where DNA mutations in several sites produce the same phenotype. Major single gene effects can be sometimes compensated in the organism using alternative metabolic pathways (McAfee, 2003).

Problems related to false positive detection of candidate genes are also common. Using crosses between two pig breeds, a polymorphism on the estrogen receptor locus (ESR) was associated to litter size in pigs with 1.5 piglet advantage for homozygous sows for the beneficial allele, and where followed by immediate recommendations for commercial use and patenting (Rotschild et al. 1996). Further research however did not confirm the effect (Gibson et al. 2002; Noguera et al. 2003; Goliášová and Wolf, 2004). Different phases of linkage between the markers and the QTL could explain the fact that the effect of the ESR locus varied widely between populations (Gibson et al. 2002). Thus, very probably, despite the ESR gene is probably a plausible ‘candidate' from their inferred physiological functions (Rotschild et al. 1996), the gene involved seems to be another one, still unknown, or the effect initially observed was the product of several, interacting genes (epistasis).

Main problems related to the use of molecular genetics in the improvement of agricultural populations (Dekkers and Hospital, 2002; Dekkers, 2004; Pollak, 2005) are:

1. Direct use of a discovered QTL effect for selection across families is not possible.

2. By the time the information about the inferred genotypes is known, frequently the animals involved in the study are not available as candidates for selection, because they will be too old.

3. Advantage from within-family selection for a QTL bracketed by markers over BLUP or phenotypic selection alone is frequently low and the methodology to exploit this information for selection is complex and relatively inefficient.

4. There are statistical estimation errors, causing both false positive and false negative effects, particularly when the effect of the QTL is small.

5. There is a lack of consistency of the effect of the same QTL between studies, caused by QTL by genetic background (epistasis) of QTL by environment interactions.

6. The net economic effect of the QTL may be lower than the effect on single traits, because unfavourable effects on other traits.

7. Selection using QTL is more complex than phenotypic selection alone. QTL information (whether the information on the QTL is direct or indirect), adds to the list of traits used as selection criteria. Issues such as reduction of selection intensities and relative emphasis given to each trait, make optimal selection more difficult, with a need for adequate relative weights for the QTL, and the polygenic portions of the genetic variation for each trait at each generation (year).

8. Short-term gains due to MAS may be at the expense of medium to long-term polygenic responses for important traits.

Even with an unambiguous knowledge for the allele effects of a mayor gene on a complex trait, expected advantages from optimum use of genotyping alleles for a QTL for a multi-generation selection horizon is not always high. The polymorphism for the αs1-casein in goats has a strong effect on protein content and total protein output. The difference between homozygous for the highest and lowest effects for milk protein is approximately three phenotypic standard deviations for milk protein content (Barbieri et al.1995; Manfredi et al. 1995). Favourable alleles have frequencies lower to 0.5 in populations undergoing selection, making a very favourable case for potential gains in protein content and production from MAS using this polymorphism. Simulation studies by Larzul et al. (1997), Fournet et al. (1997) and by Manfredi et al. (1998) indicated that when an efficient ‘conventional' progeny testing selection program is underway for increased protein production, the advantages from MAS are low to moderate. Maximum possible increase on total genetic gain for protein yield was 26%. Dekkers and Hospital (2002) emphasized the overlap that exists between marker and phenotypic information for the improvement of a multi-trait goal over several generations, using MAS. A very optimistic prospect from use of MAS as well as other biotechnologies is very common in popular commercial and non-refereed publications, based on approaches based on exploiting single gene effects, without consideration to polygenic effects, economic values or time for fixation. Research shows that the real situations are far more difficult for complex traits. These traits are controlled by several genes and environmental effects (Montaldo and Meza-Herrera, 1998). Dekkers (2004) made a survey on the status of application of MAS in actual animal breeding

programs for complex traits. He concluded that initial expectations for the use of MAS were high, but the current attitude is one of cautious optimism, with a need for careful examination of alternative selection strategies, business goals and integration of molecular and other technologies. Pollak (2005) made a detailed survey on the application of DNA technology for beef cattle improvement in USA. He concluded that current contribution of the new DNA technologies for beef cattle breeding is marginal, because they are encountering logistics and mechanical issues. For genomics technologies to impact fully on the beef industry, a higher level of sophistication of the genetic tests will be needed. Tests based on the genes themselves, rather than DNA markers associated with genes, will be required (Moore and Hansen, 2003).

It is theoretically possible to predict accurately the breeding values of animals using many markers (Meuwissen et al. 2001). From this knowledge, it is possible to develop a model for *in vitro* genetic improvement of animals. This is known as velogenetics. The model involves *in vitro* selection of cells containing the desired genes the use of totipotent embryonic stem cells (ES). The procedure uses transfection of the desired genes, selection in vitro of the cells, and nuclear transfer of the desired genotypes into receptor oocytes. This approach is supposed to increase the rate of genetic improvement by obtaining many generations in a short time by avoiding rearing, reproduction and selection of 'real animals' (Kinghorn, 1998; Smidt and Niemann, 1999; Visscher et al. 2000). Selection on the basis of genomic information only, such in this *in vitro* system, even with major genes with known effects well localized, may be dangerous, because in these artificial populations, unlike in real populations, natural selection would not be allowed to act at each generation on fitness traits under real, perhaps changing, environmental conditions. Changes on economically important traits will not be evaluated directly (Dekkers and Hospital, 2002). This may potentially reduce the responses on selected traits because of genotype x environment interactions (Montaldo, 2001). This is because selection is performed in artificial conditions that may deteriorate the fitness of the population and economic response.

Using MAS for improving health in animals by reducing disease prevalence (increasing disease resistance) or increasing resilience (the ability to withstand the disease without harmful effects), for infectious or parasitic diseases has been difficult. In most cases, excepting some rare examples such as Scrapie in sheep, complete resistance could not be obtained with the manipulation of a small number of genes. For most diseases, single-gene approaches are expected to have only a partial contribution. Gene interactions are common (Kuhnlein et al. 2003).

For many diseases, heritabilities are often low. That indicates the existence of many environmental factors affecting both the probability of infection and the response of the host. In spite of responses attained using conventional selection for some traits that are used as indicators of disease, the result is not well known. The existence of contradictory results regarding associations between production and disease resistance, the complexities of immune and resistance mechanisms and the interaction with other methods of control such as vaccination, sanitation, management and chemotherapy, makes the whole issue of selecting for disease resistance more difficult, in principle, that selecting for production traits. Moreover, we know that heritable resistance or resilience to more virulent form of pathogens would be increased by natural selection. As heritabilities for survival are generally low, we know that the genetic control of disease may be very complex, making difficult to change the outcome by manipulating single genes.

There is one published result on a successful MAS selection program to reduce the prevalence of dermatophilosis, a tropical infectious disease in Zebu cattle (Maillard et al. 2003). Maillard et al. (2003) argue to have obtained a sharp reduction in clinical prevalence of the disease from 0.76 to 0.02 in a period of five years by selecting against only two type II BoLA alleles associated with a high susceptibility of the disease. The authors explained the observed change resulting from selection performed in an unknown number of animals of each sex in 1996. However, a complete description of the changes in allele frequencies and genotypes from the moment of selection and their association with the evolution of prevalence by sex is not given. Considering the possibility of environmental changes and the presence of natural selection, in the absence of a control group, it is difficult to know if the observed change is the sole result of the mechanisms invoked by the authors through MAS.

We cannot at this moment forecast precisely the future of MAS in animal selection, but it is premature to conclude that methods based on phenotypic information will be replaced by methods based solely on genomic data (Smith et al. 2003; Van Marle-Koster and Nel, 2003). An integration of both types of data with the use of more sophisticated statistical models is needed. It is far from sure that total replacement of phenotypic information with gene-by-gene information, as selection criterion is possible or even desirable in the future.

Other very important applications of genetic markers in animal improvement include the optimization of mating strategies for non-additive genetic effects (estimation and managing of inbreeding and heterosis), parentage determination, genetic characterization of diverse animal breeds and populations using studies of between and within population (breeds) diversity (Oldenbroek, 1999) and marker-assisted introgression of particular alleles (Andersson, 2001; Dekkers and Hospital, 2002).

CLONING ADULT MAMMALS

Cloning an animal is the production of a genetically

identical individual, by transferring the nucleus of differentiated adult cells into an oocyte from which the nucleus has been removed. This is known as Nuclear Transfer and is how the Dolly sheep was produced. Since the publication of the original paper on cloning (Wilmut et al. 1997), there are several other reports on adult cloned animals involving mice, cattle, cats, goats, pigs, sheep and rabbits involving the same, and other cloning techniques (Wakayama et al. 1999; Roslin Institute online, 2003).

Cloning methods

In the case of Dolly, mammary gland cells in culture from a 6-year old donor ewe, where subjected to a reduction in the concentration of serum and thus obliged to enter in a quiescent state of the cell cycle (G0). Nuclear transfers to enucleated oocytes, was followed by electrical pulses for fusion of the donor cell nucleus and oocyte membranes and activate division (Wilmut et al. 1997).

Problems

Currently there is no doubts regarding the genetic similarity of the donor and the clone in the case of Dolly, however, besides low success rates (Edwards et al. 2003), several health problems related to the technique have been described (Samiec and Skrzyszowska, 2005). Normal development of an embryo is dependent on the methylation state of the DNA contributed by the sperm and egg and on the appropriate reconfiguration of the chromatin structure after fertilization. Somatic cells have very different chromatin structure to sperm and 'reprogramming' of the transferred nuclei must occur within a few hours of activation of reconstructed embryos. Incomplete or inappropriate reprogramming will lead to de-regulation of gene expression and failure of the embryo or foetus to develop normally or to non-fatal developmental abnormalities in those that survive (Roslin Institute, 2003; Latham, 2005). These facts indicate that there is a need for studies to determine further biological consequences of cloning. Cloning has important potential applications in gene transfer procedures (Cibelli et al. 1998a; Cibelli et al. 1998b; Colman, 1999; Roslin Institute, 2003; Li et al. 2004).

Use of Cloning in animal breeding

Use of cloning in animal genetic improvement may increase the rates of selection progress in certain cases, particularly in situations where artificial insemination is not possible, such as in pastoral systems with ruminants. Currently, high costs of cloning are one of the main factors limiting their use as a technique in practical animal breeding. Clonal groups, however more uniform than full sibs, will have all differences caused by the environmental fraction of variation for measured traits, which is usually more than 50% of total variation (Van Vleck, 1981; Van Vleck, 1999).

Selection among many cloned germlines allows the use of the non-additive genetic effects. These effects are not exploited when traditional selection methods involving sexual reproduction are used in animal improvement (Visscher et al. 2000), but most of the observed genetic variation between animals is additive (Van Vleck, 1999). Advantages in terms of additional genetic progress however, seems to be only marginal from clone evaluation in selection nucleus herds (Ruane et al. 1997). Production based on clones of the best animals of the population, may allow for a one time large ‘jump' in breeding value, so the commercial animals might be very close to those in the nucleus. However, further genetic improvement must be based in the continued use of the genetic variation by selection programs.

TRANSGENIC ANIMALS

Transgenesis is a procedure in which a gene or part of a gene from one individual is incorporated in the genome of another one. Transgenic animals have any of these genetic modifications with potential use in studying mechanisms of gene function, changing attributes of the animal in order to synthesize proteins of high value, create models for human disease or to improve productivity or disease resistance in animals (Chien, 1996; Majzoub and Muglia, 1996; Houdebine, 1998; Houdebine, 2002; Murray et al. 1999; Rao, 2000; Felmer, 2004). In the early 80´s, several research groups reported success in gene transfer and the development of transgenic mice (Gordon et al. 1980; Palmiter et al. 1982; Murray et al. 1999). The definition of transgenic animal has been extended to include animals that result from the molecular manipulation of endogenous genomic DNA, including all techniques from DNA microinjection to embryonic stem (ES) cell transfer and ‘knockout' mouse production (Cameron et al. 1994). Since the early 1980s, the production of transgenic mice by microinjection of DNA into the pronucleus of zygotes has been the most productive and widely used technique. Using transgenic technology in the mouse, such as antisense RNA encoding transgenesis, it is now possible to add a new gene to the genome, increase the level of expression or change the tissue specificity of expression of a gene, and decrease the level of synthesis of a specific protein. Removal or alteration of an existing gene via homologous recombination required the use of ES cells and was limited to the mouse until the advent of nuclear transfer cloning procedures (Houdebine, 1998; Murray et al. 1999; Rao, 2000).

Transgenic methods

Microinjection of DNA and now nuclear transfer, are two methods used to produce transgenic livestock successfully. The steps in the development of transgenic models are relatively straightforward. Once a specific fusion gene containing a promoter and the gene to be expressed has been cloned and characterized, sufficient quantities are isolated, purified and tested in cell culture if possible and

readied for preliminary mammalian gene transfer experiments. In contrast with nuclear transfer studies, DNA microinjection experiments were first performed in the mouse (Izquierdo, 2001). While the transgenic mouse model will not always identify likely phenotypic expression patterns in domestic animals, there have not been a single construct that would function in a pig when there was no evidence of transgene expression in mice. Preliminary experimentation in mice has been a crucial component of any gene transfer experiment in domestic animals (Kerr and Wellnitz, 2003). While nuclear transfer might be considered inefficient in its current form, major advances in experimental protocols, can be anticipated. The added possibility of gene targeting through nuclear transplantation opens up a host of applications, particularly with regard to the use of transgenic animals to produce human pharmaceuticals. The only major technological advance since the initial production of transgenic farm animals has been the development of methods for the *in vitro* maturation of oocytes (IVM), *in vitro* fertilization (IVF) and subsequent culture of injected embryos prior to transfer to recipient females (Houdebine, 1998; Murray et al. 1999; Rao, 2000; Wall, 2002). Another highly efficient technique for transgenesis has been recently developed based on the use of lentiviral vectors to transform cow and pig oocytes (Hofmann et al. 2003; Hofmann et al. 2004). These vectors are more efficient than microinjection in terms of transformation and expression rates. One limitation is that the size of the transgene and the internal promoter has to be less than 8.5 kb in size.

TRANSGENESIS IN THE IMPROVEMENT OF PRODUCTION TRAITS

The technology of transgenesis is potentially useful to modify characters of economic importance in a rapid and precise way. Contrary to the 'classical' selection programs, it is necessary a knowledge of the genes that control these characters and their regulation.

Following is a brief discussion of experiences with transgenesis to alter economically important traits in livestock.

Growth and meat traits

In most of the earlier work in domestic species (pig, sheep, rabbit) growth hormone was enhanced by the metallotionein promoter to control its expression. Subsequent efforts to genetically alter growth rates and patterns have included production of transgenic swine and cattle expressing a foreign c-ski oncogene, which targets skeletal muscle, and studies of growth in lines of mice and sheep that separately express transgenes encoding growth hormone-releasing factor (GRF) or insulin-like growth factor I (IGF-I) (Palmiter et al. 1982; Cameron et al. 1994; Murray et al. 1999). Transgenic pigs and sheep with high levels of serum growth hormone were obtained, but an increment of its rate of growth was not observed, and only in some lines average daily gain increased with the supplement of the diet with high levels of protein. The highest effects were observed in the reduction of body fat. A large number of different serious pathologies and a severe reduction in reproductive capacity were described in these animals (Murray et al. 1999). In a report about two studies with pigs (Neimann, 1998), there is evidence for the use of transgenesis allowing to important reductions in body fat and increased diameter of muscle fiber by increased IGF-I levels and growth hormone without serious pathological side effects. Australian regulations avoided the commercial release of these animals.

Frequently the used promoters have not allowed an efficient control of the expression of the transgene. It was assessed that it is necessary to develop more complex constructions that activate or repress the expression of the transgene more precisely. Adams et al. (2002) found inconsistent results regarding the effect of a growth hormone construct in sheep on growth and meat quality.

Recently, a spectacular transformation was obtained by insertion of a plant gene in pigs. Saeki et al. (2004) generated transgenic pigs that carried the fatty acid desaturation 2 gene for a 12 fatty acid desaturase from spinach. Levels of linoleic acid (18:2n-6) in adipocytes that had differentiated in vitro from cells derived from the transgenic pigs were 10 times higher than those from wild-type pigs. In addition, the white adipose tissue of transgenic pigs contained 20% more linoleic acid (18:2n-6) than that of wild-type pigs. These results demonstrate the functional expression of a plant gene for a fatty acid desaturase in mammals, opening up the possibility of modifying the fatty acid composition of products from domestic animals by transgenic technology.

Wool production

The objectives are to improve production of sheep wool and to modify the properties of the fiber. Because cystein seems to be the limiting amino acid for wool synthesis, the first approach was to increase its production through transfer of cystein biosynthesis from bacterial genes to sheep genome (Murray et al. 1999). This approach did not achieve the efficient expression of these enzymes in the rumen of transgenic sheep.

Milk composition

Milk proteins are coded by unique copy genes that can be altered to modify milk composition and properties. Among the different applications of milk modification in transgenic animals (Maga and Murray, 1995; Murray et al. 1999), the following can be highlighted:

1. To modify bovine milk to make it more appropriate to the consumption of infants. Human milk lacks β-lactoglobulin, has a higher relationship of serum proteins to caseins, and has a

higher content in lactoferrin and lysozyme when compared to bovine milk. Lactoferrin is responsible for the iron transport and inhibits the bacterial growth. To introduce the human lactoferrin into the bovine milk, transgenic cows have been obtained (Van Berkel et al. 2002). The elimination of the β-lactoglobulin in the cow milk would be another interesting objective because is one of the major allergens of cow's milk.

2. To reduce the content of lactose in the milk to allow their consumption to people with intolerance to lactose. It is considered that 70% of the world population is lacking theintestinal lactase, the enzyme required to digest the lactose. The reduction in lactose may be obtained by expressing β-galactosidase in the milk or diminishing the content of α-lactalbumin. Transgenic mouse with inactivated α-lactalbumin gene produce milk without lactose. However, a serious practical drawback of this method is that this milk is very viscous and it is not secreted to the exterior of the mammary gland, due to the importance of the lactose in the osmoregulation of the milk (Stinnakre et al. 1994).

3. To alter the content of caseins of the milk to increase their nutritive value, cheese yield and processing properties. Research has intended to increase the number of copies of the gene of the κ-casein, to reduce the size of the micelles and modificating the κ-casein to make it more susceptible to the digestion with chymosin. This has only been done using the mouse as a model (Gutiérrez-Adan et al. 1996). Brophy et al.(2003) engineered female bovine foetal fibroblasts to express additional copies of transgenes encoding two types of casein: bovine β-casein and κ-casein. The modified cell lines of fibroblasts were used to create eleven cloned calves. Milk from the cloned animals was enriched for β- and κ-casein, resulting in a 30% increase in the total milk casein or a 13% increase in total milk protein, demonstrating the potential of this technology to make modified milk.

4. To express antibacterial substances in the milk, such as proteases to increase mastitis resistance. The objective is to alter the concentrations of antibacterial proteins such as lyzozyme or transferrin in the milk (Kerr and Wellnitz, 2003; Felmer, 2004).

Future perspectives of transgenesis

The techniques for obtaining transgenic animals in species of agricultural interest are still inefficient. Some approaches that may overcome this problem are based on cloning strategies. Using these techniques it is feasible to reduce to less than 50% the number of embryo receptor females, which is one of the most important economic limiting factor in domestic species. It would also facilitate the further proliferation of transgenic animals. Recent results relate these techniques with still low success rates (Edwards et al. 2003), high rates of perinatal mortality and variable transgenic expression that requires to be evaluated before generalizing their application (Houdebine, 2002; Samiec and Skrzyszowska, 2005).

Considerable effort and time is required to propagate the transgenic animal genetics into commercial dairy herds. Rapid dissemination of the genetics of the parental animals by nuclear transfer could result in the generation of mini-herds in two to three years. However, the existing inefficiencies in nuclear transfer make this a difficult undertaking. It is noteworthy that the genetic merit of the ‘cloned' animals will be fixed, while continuous genetic improvements will be introduced in commercial herds by using artificial insemination breeding programs (Karatzas, 2003).

In an alternative scenario of herd expansion, semen homozygous for the transgene may be available in four to five years. Extensive breeding programs will be critical in studying the interaction and co-adaptation of the transgene(s), with the background polygenes controlling milk production and composition. Controlling inbreeding and confirming the absence of deleterious traits so that the immediate genetic variability introduced by transgenesis is transformed into the greatest possible genetic progress is equally critical (Karatzas, 2003).

Another alternative strategy for transgenesis is based on the use of sperms as vectors in the integration of the transgenes. Initially described in mice (Lavitrano et al. 1989). Results showed that this procedure might be efficient in sheep (Niemann, 1998). In addition, a successful expression of a gene related to genetic modification of pigs for a gene related to xenotranplantation was obtained using this technique. Eighty percent of the pigs were transformed and 54% expressed the transgene consistently (Lavitrano et al. 2002). A very efficient modification of this technique that uses the co-injection of sperms and DNA, has been described in the mouse and given a high rate of transgenesis (20%), therefore, their application to domestic species seems promising (Perry et al. 1999; Wall, 2002). Intracytoplasmic sperm injection (ICSI) has been used recently for the stable incorporation and phenotypic expression of large yeast artificial chromosome (YAC) constructs of submegabase and megabase magnitude. This technique allowed for more than 35% of transgenesis (Moreira et al. 2004). Another option for transgenesis is the use of insertional mutagenesis using natural transposons. A transposon system called “Sleeping Beauty”, and active in a wide range of vertebrate cells, was used to transform mouse embryos with mRNA expressing the SB10 transposes enzyme (Dupuy et al.

2002). Kuroiwa et al. (2004) targeted sequentially a system for primary fibroblasts cells that were used to knock out both alleles of a silent gene, the bovine gene encoding immunoglobulin-μ (IGHM), and the active gene encoding the bovine prion protein (*PRNP*) and produced both heterozygous and homozygous knockout calves. The procedure integrates homologous recombination to replace genes in cell culture, and rejuvenation of cell lines by production of cloned fetuses. A method for selective elimination of selection marker genes was also developed. This method allow for the production of double homozygous transgenic embryos in 21.5 months. In contrast, for cattle, the production of double homozygous from heterozygous founders would require approximately 5 years and generation for double homozygous from heterozygous founders is impractical. This method can be used to breed many types of cattle with improved disease resistance and values for increased productivity. A recent alternative consists on the transformation of somatic tissues of developed animals, using techniques similar to those used in gene therapy (Kinghorn, 2003).

DISCUSSION

Detecting genes related to disease and their expression in humans from studies on the genome, could lead to the development of therapies and the development of drugs for specific individuals, and enhanced early diagnosis of individuals with high-risk genotypes, allowing for preventive or remedial actions, even gene therapy. In animals, this knowledge could lead, in addition, to select against defective genes.

In livestock, knowledge of effects of specific genes and gene combinations on important traits could lead to their enhanced control to create new, more useful populations. The use of specific gene information is not a panacea, but could help to increase rates of genetic improvement, and open opportunities for using additive and non-additive genetic effects of domestic species, provided wise improvement goals are used and this new technology is optimally used together with the so called ‘traditional' or ‘conventional' methods based on phenotypic and genealogical information.

These methods will help to increase our knowledge about the genetic architecture of complex quantitative traits in domestic animal populations and to estimate the distribution of the genetic variation across and within breeds and population. It will also aid in ascertaining the genetic merit of local, less known populations (Hill, 2000). Studies for using genetic diversity in structured populations using DNA markers (Hartl and Clark, 1997) are very useful in order to set priorities for conservation of distant or unique populations as reservoirs of potentially unique genes, because their contribution to biodiversity would be greater (Oldenbroek, 1999). Currently, however, the main practical application of DNA markers is for parenting determination and to trace products such as meat (Kinghorn, 2003; Pollak, 2005).

Despite its relatively low success rates and associated high costs, transgenic technology have a number of important potential applications in animal improvement such as increasing productivity, product quality and creating novel products.A major limitation to use transgenesis in the improvement of productive characters is the limited knowledge available on the identity and regulation of the genes that control these characters. The advance in the elaboration of genetic maps and fine positional cloning studies in the main species of interest will allow having a larger number of candidate genes susceptible of being manipulated. However, the road from genotype to phenotype is proving to be much more complex than previously thought for disease and production traits affected by many genes (True et al. 2004).

One promising applications of transgenesis is the synthesis of biomedical products of high commercial interest. Transgenic bioreactors and the use of exogenous or artificial genes interfering with particular cell mechanisms or with pathogens but not, or only marginally, with the physiology of the animals are potential applications. A greater knowledge on the mechanisms that determine the integration of the transgenes and genic regulation will allow a more precise control of the expression of the transgenes and it will probably facilitate a larger number of applications in the domestic species, including modifications beyond normal limits, such as to increase the number of copies of the gene and their expression. These transformations could be regarded as a form of mutation (Hill, 2000). The expressions of complex traits are the result of several mechanisms involving both regulatory and structural portions of the genome (Schutze, 2004; Whitelaw et al. 2004). Advances in molecular genetics, genomics, proteomics and transcriptomics (Dunwell et al. 2001; de Hoog and Mann, 2004; Honore et al. 2004) might perhaps help to shorten the gap between the more ‘holistic' approaches of quantitative genetics with the more ‘reductionistic' approach of molecular genetics. The release of genome sequence information in cattle (Sonstegard and Van Tassell, 2004) and pig (Wernersson et al. 2005), may allow for a more efficient use of MAS and also to address some consumers concerns regarding product quality and safety.

Use of genetic engineering for animal and plant improvement is in its infancy, therefore many questions regarding efficiency, safety and societal benefits in particular situations remain. Problems arising transgenic plants, including their lower-than expected productivity, are reviewed thoroughly by McAfee (2003). Simplistic and overoptimistic views of biotechnology should be replaced by serious and scientifically based assessments of these new technologies by potential users on a case-by-case basis. We need to emphasize that in most cases, the use of MAS is not a revolution but just an evolution with regard to the traditional methods, because we are looking to improve

more efficiently traits that already are actually or potentially improved in an efficient way using, for instance, mixed model (BLUP) based technologies for selection. Efficiency issues are very important. In order to increasing the efficiency of MAS, we need previously to:

1. Define with greater precision the selection goal and selection criteria (Monin, 2003).
2. Optimize the use of BLUP and other 'classical' breeding methodology.

The use of transgenic animals models for the study of gene regulation and expression has become commonplace in the biological sciences. Contrary to the early prospects related to commercial exploitation in agriculture, there are some challenges regarding their use that still lay ahead (Archibald and Haley, 2003; Sang, 2003; Sillence, 2004). The risks at hand can be defined not only by scientific evidence but also in relation to public concern (whether perceived or real) that exists in some people (Larrère, 2003). Therefore, the central questions will revolve around the proper safeguards to employ and the development of a coherent and unified regulation of the technology.

Cloning is another technique that raises concerns both from the ethical and practical point of view. Whether it is acceptable to clone humans is a very difficult issue. In animals, besides the very low success rates, some abnormalities should suggest that more information is required on the consequences of such practices in humans but also in animals, before its routine use. Advantages for animal breeding programs derived from cloning with no use of transgenesis are like to be small (Van Vleck, 1999).

These two examples illustrate that in spite most of the problems are technical in nature, implications of the use of this knowledge will be important for the society as a whole (Olsson and Sandoe, 2004).

A reasonable degree of regulation, open information on the issues of genetic engineering technologies from the academic world and an involvement of the whole society in the developments of the laws concerning these issues, seems to be the best way to circumvent an exaggerated or negative reactions to some of these knowledge, and to avoid or reduce unethical or abusive use of these techniques (Fukuyama and Stock, 2002). A specific set of conclusions regarding safety of food from genetically modified animals is available from a FAO/WHO expert consultation panel (FAO/WHO, 2003).

CONCLUDING REMARKS

Most of the important potential technical advances offered by genetic engineering technology in animal breeding are still ahead. Their use has both advantages and problems. Advantages are related to a more complete control over the animal genome. Problems are related to technical complexity, high costs, in some cases, public acceptance and ethical dilemmas.

It is not likely that this technology, will replace 'conventional' methods for genetic improvement. Instead, they probably will begin to be gradually incorporated into current genetic improvement programs that use efficiently classical improvement methods to achieve particular objectives.

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