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Genetics then and now: breeding the best and biotechnology

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Summary

In the past, domesticated animals were genetically improved by identifying meritorious individuals, mating animals displaying desired traits, continued breeding of related animals to perpetuate their superior traits and cross-breeding when inbreeding depression became evident. Today, assisted reproduction and biotechnology allow breeders to design and direct the reproductive course, disseminate desired traits and hasten genetic improvement. Generation interval can be greatly reduced by combining artificial insemination, which is the oldest and most widely used assisted reproductive technology, with the more recent techniques, such as oestrus synchronization, superovulation, ovum pick up from immature females even out of breeding season, and *in vitro* embryo production and transfer. Furthermore, the sex and genetic make-up of the offspring can be selected by using sex-sorted sperm for insemination, marker-assisted selection, functional deletion or addition of specific genes to the offspring's genome, or somatic cell nuclear transfer for cloning. However, the poor success rates with some of these procedures have delayed their large-scale application which, in turn, has hindered the proper evaluation of their genetic impact. The potential genetic consequences of some of these approaches merit the same degree of diligent evaluation that is currently extended to the procedures used for overcoming their 'technical' inefficiencies.

Keywords

Assisted reproductive technology – Genetic improvement – Inbreeding – Marker assisted selection – Transgenesis.

Introduction

Domestication of food animals and companion animals has inevitably led to efforts to control their reproduction. In the case of food animals, the objective of reproductive control has always been to increase the yield of milk, meat, wool and other commodities useful to man. Cattle were originally domesticated to provide milk, meat and labour to their owners (20). More recently, breeders have concentrated on the evolution of dual purpose (milk and meat) or single purpose (milk or meat) breeds, but the primary objective remains to create more offspring regardless of the target product. With the advent of new technologies in recent years, breeders have sought to make the reproductive programme more efficient by manipulating the breeding season, reducing the generation interval by lowering the age at which breeding commences, stretching the reproductive lifespan and increasing the frequency of breeding. Breeding strategies in companion animals have emphasised the perpetuation of superior skills and other 'desirable' traits. These goals are achieved using selective breeding.

While in the past selective breeding accomplished through 'natural' means required a long time to make perceptible changes, today, selective breeding is aided by various assisted reproductive technologies (ARTs) combined with advanced biotechnological approaches. This paper reviews the avenues currently open to breeders to increase the rate of genetic

progress. The potentially negative impacts of some of these approaches to shorten generation intervals and produce more offspring from high-merit animals are discussed in the light of current issues affecting the cattle industry. Although other food animals are mentioned in relation to the application of specific techniques, the bulk of the work reviewed concerns cattle, which are the most numerous and the largest of the food animals and, in terms of genetics, the most exhaustively studied domestic animals. Cattle also serve as models for the application of ARTs in other domesticated ungulates, and the development of newer approaches is largely driven by the prospect of commercial benefits in cattle farming.

Domestication

Skeletal remains dating back to 6500 BC indicate that the domestication of cattle started around 10,000 years ago (19). It is generally accepted that the domestication of cattle followed that of sheep, goats, pigs and dogs and that both subspecies of domesticated cattle (*Bos indicus*, native to India, and *Bos taurus*, the European cattle) evolved from a common ancestor, the wild ox (20). Geographically distinct strains of the wild ox (aurochs; *Bos primigenius*) were found in Eurasia and parts of North Africa until they became extinct in the early 17th Century (20). Domestication, which may have originally involved just a few captured wild oxen, is believed to have occurred repeatedly, at different times, in different places and with widely varying ancestral strains. The differences in the appearance of present-day cattle populations and their division into the various 'subspecies' broadly included in the *B. indicus* and *B. taurus* clades, are thought to reflect differences in the timing of domestication and the strains of aurochs used for domestication in different parts of the world. In addition, the lack of subsequent hybridisation between different geographical isolates may have helped to perpetuate and preserve the distinct traits reflected in the groups of animals referred to as breeds (97), although breed registries and standards for different breeds were introduced only relatively recently.

Establishing a breed or stock in the old days must have been a complicated and onerous task, involving the recognition of meritorious animals, based on a desired phenotype (type, colour, temperament, performance, etc.), and selective breeding to perpetuate their genotype (i.e. the gene or genes responsible for the desired traits). The traits selected for would have been those that made the animals more amenable to domestication, including docility and smaller size (to allow their owners to handle them) and the ability to survive nutritional, climatic and disease-related stresses. The phenotypes selected later would have included prolificacy, early maturity, coat colour, length and type of horns and attributes associated with the ability to produce useful commodities such as milk, meat, hide and dung. After a few generations of selection for one or more of these traits, a herd or flock would be established in which most of the animals exhibited the desired phenotype and were genetically related.

Breeders would have continued to breed close relatives among their own stock, were it not for the risk of degeneration that threatens such painstakingly created herds. The problems with breeding relatives were known to animal breeders before the concept of genetics became widely understood (14). The rediscovery of Mendelian principles of factorial inheritance early in the last century (14) unmasked the potential of genes to eventuate diseases and disorders and revealed the genetic principles underlying the undesirable consequences of breeding relatives. The increased frequency of defective animals and the decreased reproductive efficiency associated with continued breeding of close relatives of any species (inbreeding) were soon recognised as an unwelcome consequence of parents imparting identical alleles for any given locus, thereby increasing the proportion of homozygous progeny (34, 77, 78). Thus, inbreeding, which fixes a trait in a herd by bringing the genes controlling that trait together, also increases homozygosity at other loci, for both desirable and undesirable genes. Inbreeding *per se* does not create mutant genes, but it helps to bring together mutant genes already present in the herd, so that their negative impact can manifest as reproductive problems or birth defects. The problems encountered in closely inbred herds, therefore, are attributed to the increase in the probability of the progeny receiving the same mutant gene from both parents. This probability is referred to as the inbreeding coefficient (78, 82).

If inbreeding has resulted in a relatively high level of homozygosity, selective breeding will be less effective and further genetic improvement will be difficult (78, 98). When such a stage is reached, the breeder has to bring in new genes from another source (or from several sources) or start all over again (79). Approaches used by animal breeders to avert the problems caused by inbreeding include line-breeding, which is a general term for mating relatives that are most distantly related. Mating the progeny with a relative of the previous generation (line-breeding) delays reproductive breakdown (82). However, mating totally unrelated animals (intraspecific, interspecific or intergeneric out-crossing) has varying effects on reproductive potential, ranging from improved reproductive function to total or partial sterility, with males often being more severely affected (9, 79).

Genetic basis of reproductive problems

Reproduction, regardless of the species, breed or sex of the individual, is a complex phenomenon. As such,

reproductive processes can be interrupted by genetic or environmental factors at any stage in gametogenesis, during transportation of the gametes to the site of fertilisation or during the foetal, perinatal or neonatal growth phases. Advances in human genetics during the last century and the recognition that many reproductive problems may have a genetic basis have encouraged animal geneticists and veterinarians to examine the possible role of genes in reproductive defects in domestic animals. A variety of mutations that affect fertility by interfering with specific processes in reproduction, from gametogenesis to the viability of the newborn, have been reported by animal geneticists (78, 82, 127, 132); these include the gene mutations leading to anatomical malformations that prevent reproduction, cause abnormalities of the gametes leading to infertility, elicit maternal-foetal conflicts leading to embryo or foetal mortality and interfere with reproduction and other apparently unrelated phenotypic features including coat colour and horn development (34, 44, 68, 79, 90, 132).

Knowledge of the causes of reproductive problems was substantially increased during the latter half of the last century when chromosome analysis allowed the (chromosomal) integrity of breeding animals to be tested. Such analysis, together with pedigree evaluations, helped to establish the genetic aetiology of reproductive problems, which can be roughly classified into three categories: defects caused by mutations at a single locus (monogenic defects); defects caused by mutations at more than one locus (polygenic defects); and defects caused by changes in the number and/or structure of the chromosomes (chromosomal defects) (10, 64, 106, 107).

The complex physiological processes involved in reproduction, including those that can be interrupted by a mutant gene or genes, can now be elucidated at the genetic level thanks to the development of molecular biology techniques over the past few decades. However, prior to the advent of molecular biology, breeding data and pedigree analysis were the only means of tracking the pattern of transmission for many of the inherited reproductive problems of domestic animals. Even though reproductive efficiency has long been recognised to be multifactorial and of low heritability (78, 82, 127), many of the defects interfering with specific steps in the reproductive process were recognised as single-gene (Mendelian) traits and were considered to be dominant or recessive (autosomal or sex-linked) based on the pattern of transmission revealed by pedigree analysis (10, 11, 13, 78). Since many of these defects occur in the progeny of related parents, a majority of the traits encountered were reported to be autosomal recessive (65, 82). However, the recurrence pattern of some of the birth defects and metabolic errors was sporadic, albeit familial, and did not conform to the expectations for monogenic defects (106, 107). It was realised that such malformations and metabolic errors result from homozygosity for mutant genes at various loci leading to a high concentration of defective genes in the conceptus (13, 106, 107). Carriers of these genes are generally undetected until several generations of inbreeding have occurred. With each generation, the potential for further concentration of bad genes is increased, and the liability of the conceptus to specific diseases or disorders is also increased. These genetic defects, resulting from the concentration of 'liability genes' beyond the threshold that permits the birth of a 'normal' offspring, are referred to as 'threshold traits' (106, 107). The majority of the defects encountered in domestic animals, especially those more frequently seen in highly inbred strains, belong to this category (13, 106, 107). High concentrations of defective genes (rendered homozygous at various loci by inbreeding) are not compatible with viability, and often lead to the death of the conceptus at the embryonic, foetal or neonatal stage. Since such deleterious genes (referred to as lethal genes) invariably eliminate the homozygotes (and sometimes also the heterozygotes), inbreeding is also viewed as a means of purifying a stock. However, a proportion of good genes, often linked to bad genes, are lost along with the bad genes when an animal dies (78, 82).

Chromosome defects and reproduction

Various types of chromosome abnormalities have been implicated in the reproductive problems of domestic animals including cattle (10, 114). The cattle karyotype consists of 29 pairs of acrocentric (single-armed) autosomes and a pair of submetacentric (bi-armed) sex chromosomes. The autosomes, which are conventionally arranged in decreasing order of length in the karyotype, range in percent total complement lengths (%TCL) from 5.87 to 1.99, while the X and Y chromosomes account for 5.45 and 2.13 respectively, in %TCL (48, 115). Bovine sex chromosomes are easy to distinguish from the autosomes; however, bovine autosome pairs are not easily identified on the basis of differences in length alone. The introduction of chromosome banding techniques has alleviated this difficulty to some extent and made it possible to visualise segmental details of individual chromosomes as specific banding patterns (114). Today, each autosome pair can be identified by its 'marker' gene image visualized with fluorescent *in situ* hybridisation (FISH) using mainly biotinylated cosmid probes and fluorochrome coupled avidin for signal detection (96, 115).

Various chromosome rearrangements of the Robertsonian type (translocation of the whole arm of an acrocentric chromosome to the centromere region of another acrocentric chromosome) have been identified in cattle

displaying different degrees of reproductive problem (114). More recent studies, using combinations of banding techniques and FISH, have uncovered over 45 different Robertsonian translocations in cattle breeds from various countries (48). Of these, the Robertsonian translocation involving the longest (chromosome 1) and the shortest (chromosome 29) chromosomes (rob.1/29 T), referred to as Gustavsson's anomaly, is the most widely distributed in cattle populations worldwide (10, 64, 114). Gustavsson's anomaly is also the most exhaustively studied chromosome rearrangement in terms of its impact on reproduction (64, 114). Other translocations involving different pairs of autosomes and other types of structural interchange between pairs of non-homologous chromosomes or between an autosome and an X chromosome (reciprocal translocations) have also been reported in cattle and other domestic animals (12, 48, 106, 107). All these translocations interfere with gametogenesis and moderately or severely reduce fertility in the carriers, even though the individuals appear phenotypically normal (11, 12, 48). For example, rob.1/29 T reduces fertility by 5% to 10% through embryonic and foetal mortality arising from the chromosomally unbalanced gametes produced by carriers (40, 48, 89). This type of reproductive problem in carrier bulls and cows has led to the establishment of eradication programmes (based on karyotyping bulls before they are imported and culling detected carriers before they enter the testing programme for artificial insemination) in Sweden and various other countries (48, 64). Identified carriers are generally not maintained for breeding because of their lowered reproductive performance. Indeed, the different types of translocations reported in cattle (48) belong to the category detected in one or more carrier bulls or cows destined for the slaughter house because of their poor fertility. Exception to this trend is rob.1/29 T, which is widely distributed in the cattle populations of the world. This Robertsonian translocation is thought to have conferred some, as yet unidentified, beneficial effects on carriers under previous production conditions (12, 48, 114).

During the second half of the last century, the criteria developed for selecting bulls (and, to a lesser degree, for dams) included increased milk yield and improved milk composition, udder health, fertility, calving ease, body weight, feed intake, milking speed, temperament and herd lifespan of the progeny of the daughters and granddaughters, in addition to specific semen characteristics for rating bulls for dairy breeds (63). While these desirable traits were used to select animals for breeding, information on the reproductive impact of chromosome defects and gene mutations enabled breeders to decide which animals were to be excluded from the breeding programme (40, 48, 89). Today, some of the reproductive problems caused by genetic or chromosomal defects can potentially be overcome by using biotechnology and ARTs. However, since some of these technologies themselves can accelerate the accumulation of bad (as well as good) genes in a herd or flock, an examination of their merits, in the light of their genetic impacts on the species, is important.

Artificial insemination

Of the currently used ARTs, artificial insemination (AI) is the first and most important means of achieving genetic improvement. However, it is also one of the most effective causes of genetic erosion in farm animals. It is well recognised that AI has revolutionised the animal breeding programme in general and has contributed significantly to the genetic improvement of cattle in particular (16, 47). This procedure, which involves collecting semen from males and using it to impregnate females, has been used since the beginning of the 20th Century and has been applied in other mammals, including dogs, foxes, rabbits and poultry, in different parts of the world (47, 81). As a means of effecting genetic improvement in dairy cattle, AI has been in use for over 65 years (16, 47). Although the procedures currently used for collecting and handling semen are different from the original procedures and are still being refined, AI in one form or another is an integral part of all the other ARTs developed more recently. The success and popularity of this technique are the result of the establishment of methods for identifying males of the highest genetic merit and of the criteria developed for semen characterisation (17, 93). Thus, semen donors are selected from males classified as meritorious on the basis of a combination of parameters for progeny testing, especially in the dairy cattle industry, where the progeny criteria are rigorously defined (47). The method of semen processing (fresh, refrigerated or frozen) and the site of semen deposition (vaginal, cervical or intrauterine) depend on the specific situation and the species of domestic animal inseminated (7, 55, 102). The use of refrigerated semen (stored at approximately 4° C and used within 24 hours of collection) was popular with breeders of cattle and small ruminants at one time, because a specific male could be shared by a group of breeders within the distance that could be covered by the inseminator (47). The introduction of cryoprotective agents and the recognition that glycerol has cryoprotective properties for mammalian spermatozoa (130, 131) have enormously widened the application of AI for the genetic improvement of domestic animals. The use of frozen semen for AI has also made it possible for genes to migrate from one population to another through the marketing of male germplasm, for the females of seasonal breeders, including sheep and goats, to be bred during the non-breeding season, and, most importantly, to preserve and use the germplasm of a meritorious male beyond his reproductive lifespan (7, 47, 55).

Various modifications were made to the AI procedure after it was discovered that physiological alterations of sperm can affect fertilisation (3, 27, 28), and methods are still being developed to prevent or reduce sperm deterioration through early capacitation and acrosome reaction (25, 26, 47). Recent additions to the conventional AI procedure include flow cytometric semen sorting to obtain 'X-enriched' and 'Y-enriched' sperm fractions which can be used to select the sex of the progeny (123, 124, 136), the introduction of a heterospermic index as a tool to test the fertility potential of bulls (46) and the identification of the beneficial effects of seminal plasma (SP) as a resuspending medium for frozen-thawed semen (43, 76, 101, 102). Using SP for suspending frozen semen increases the pregnancy rates in inseminated female ungulates by preventing damage to the sperm membranes (43, 76, 102). Further increases in pregnancy rate followed the identification and use of SP components, including osteopontin and immunoreactive relaxin, to reduce the damage to sperm membranes during freezing and handling of semen (88, 91).

Sex selection

Selecting the sex of the progeny by using sex-sorted sperm for AI has long been a dream of dairy cattle breeders, as males are of limited commercial value to them. Predetermining the sex of the offspring has also been a goal of investigators in various fields, including other components of agriculture, human medicine and wild-animal conservation biology. Sex selection could reduce the incidence of sex-linked diseases in humans, breeders of farm animals could 'order' individuals of a chosen sex according to need, and conservation biologists could use sex selection as an effective strategy in the re-population of endangered species. The sperm-sexing technology referred to as 'Beltsville sperm sex sorting' is currently thought to be the most popular method for breeding domestic animals (76, 102, 123, 124, 125, 136). This approach uses flow cytometry to separate spermatozoa carrying fluorescent-labelled X chromosome from the spermatozoa carrying fluorescent-labelled Y chromosome. Continued improvements in instrumentation and refinements of sample handling have resulted in this technique now being capable of sorting 15 million spermatozoa per hour into X-carrying and Y-carrying lots (123, 125). This approach has been used successfully in cattle (125), sheep (76, 102), horses (24) and pigs (118). Even though the number of spermatozoa obtained after sorting tends to be low, acceptable pregnancy rates can be obtained if *in vivo* insemination takes place close to the expected time of ovulation and the sorted sperm are deposited deep in the uterus (102). Combining this method with other ARTs has resulted in the birth of offspring of the desired sex in a wide range of domestic animals, including dairy cattle in which sperm sexing is of considerable commercial value (123, 136). Breeding cows using sexed sperm does not lead to significant differences in gestation length, birth weight, calving ease, calf vigour, weaning weight, abortion rate or neonatal death rate (136). Furthermore, 87.8% of calves born from X-sorted sperm are heifers and 92.1% of calves born after insemination with Y-sorted sperm are bull calves, giving a sex-sorting accuracy of approximately 90%, compared with the 50.8% heifers and 49.2% bull calves obtained from unsorted sperm (123, 136).

One of the lingering limitations of this approach is that, since flow cytometric sorting is carried out one sperm at a time, the number of sexed sperm produced per unit of time is limited, and those that are sorted display a variety of changes, including destabilisation of the sperm membrane and capacitation-like changes, that reduce the lifespan of sorted spermatozoa in the female genital tract (136). Although fertilisation of bovine oocytes is reported to have occurred even with dead spermatozoa (60), the loss due to fertilisation failure and poor-quality embryos in superovulated cows inseminated with sex-sorted sperm is much higher than in cows inseminated with sperm that have not been sex-sorted (120). Despite these limitations, the technology is now being used commercially in cattle, with inseminations accomplished with relatively few sperm (123). Recent studies indicate that the fertilising lifespan of sex-sorted sperm can be extended by re-suspending the sorted fraction in SP or SP-components, which reduce the membrane destabilisation and capacitation-like changes sustained by spermatozoa during sorting, freezing and thawing (25, 26, 88, 102).

Although these aspects of AI are still being refined, it should be noted that none of the ARTs available today would have been possible if AI were not already available as a vital component of these approaches. Reproductive technologies such as superovulation, embryo transfer, *in vitro* fertilisation (IVF), *in vitro* embryo production (IVP) and cloning rely on the efficient use of AI, and all of these technologies are constantly being refined for eventual use in routine animal breeding.

Manipulation of oestrus and ovulation, and embryo transfer

Hormonal manipulation of females to induce superovulation (multiple ovulation) prior to insemination combined with transfer of retrieved embryos into hormonally primed surrogates is often regarded as the female counterpart of AI, since both approaches aim to produce more offspring from genetically valuable individuals (94). However, the techniques differ in the maximum number of offspring that can be created. Embryo transfer techniques have been applied to various

mammals and have undergone various modifications over the last century (1, 66, 67, 137), and, in some countries, live (and sexed) calves have been produced using this technique since the 1950s (71). However, it emerged as a popular and feasible method for disseminating superior female genes in cattle breeds only after the introduction of non-surgical embryo retrieval and transfer (16, 133). Non-surgical retrieval and transfer have now been used successfully in a variety of domestic animals, and embryos have been exchanged between countries since the 1970s (16, 74, 109, 122, 147). Superovulation can be used to increase the number of animals carrying genes from a female already proven to be of superior production value, and it is also an important tool for obtaining embryos for immediate transfer, cryopreservation (for later use in transfer) or research. However, the response to hormonal manipulation in small ruminants is variable, with 25% to 50% of donors (among goats) not responding (7, 86). Furthermore, the quality and number of transferable ova retrieved from the 'responders' vary greatly depending on the breed, age, nutritional plane and other factors contributing to early regression of corpora lutea or to poor fertilisation due to interrupted sperm transport to the ova (7, 86).

Induction of multiple ovulation followed by embryo transfer (often referred to as multiple ovulation and embryo transfer, or MOET) has been proposed as a way of establishing nucleus breeding herds for the purposes of accelerating genetic improvement (106, 107, 109). MOET reduces the generation interval and has been reported to increase the rate of genetic improvement by approximately 30%, compared with that achieved through conventional breeding schemes involving 'progeny testing' (139). This approach is used in the breeding of beef cattle to import and export valuable genetic material and to increase the number of newly imported exotic individuals much more quickly than can be achieved through natural reproduction. In the dairy cattle industry, MOET is used to propagate elite stud animals as well as to export valuable genes (*via* the embryos) from one country to another. However, MOET is not used routinely in the genetic improvement of dairy cattle because of the cost, the need for technical sophistication and, more importantly, the inconsistency of the results. In contrast, hormonal synchronisation of oestrus and ovulation has made a major difference to the overall efficiency of the assisted reproductive programme (16). Oestrus synchronisation, together with AI, is widely used in the breeding of beef and dairy cattle and small ruminants (16, 35). Combining oestrus synchronisation with *in vitro* maturation (IVM) and fertilisation of oocytes, culture procedure for embryo production, and cryopreservation, allows offspring (and milk) to be produced at times of the year outside the normal season for some domestic animals, including sheep and goats.

An improvement over standard oestrus synchronisation is ovum pick-up (OPU), either by laparoscopy in small ruminants or by transvaginal ultrasound-guided retrieval in cattle (5, 6, 45, 80, 117, 129). Ovum pick-up can be repeated several times a week during the reproductive life of a genetically valuable female, at any time of the year (regardless of the season in seasonally breeding females) and at any time suitable for the breeder or technologist, since no prior hormonal stimulation is required (45, 80, 117, 129). In combination with IVP, OPU can increase the number of offspring that can be produced from genetically valuable females because it allows a consistently large number of ova to be retrieved from a known donor. Furthermore, since OPU can be applied to pre-pubertal heifers and juvenile does and ewes, progeny can be obtained long before the individuals could produce offspring through natural breeding (7, 33, 117).

Transgenesis

A component of biotechnology that has impelled genetic progress in areas that were traditionally achieved only by cross-breeding is transgenesis (105). Transgenesis involves introducing specific genes into the genome, thereby ensuring their stable incorporation into the germ line of farm animals, and is a major scientific advance in animal agriculture. Various areas of livestock production stand to benefit from transgenesis, including those targeting reproductive performance, growth rate, carcass quality, milk production, milk composition and disease resistance (140, 143). All these attributes are polygenic traits (10, 106, 107). As such, in the earlier days of domestication, the introduction of superior alleles for any of these traits into a new line would have necessitated continued genetic selection, cross-breeding (hybridisation) and repeated back-crossing to ensure the introgression of the introduced allele. Transgenesis offers a faster method of introducing new and desirable genes into domestic animals without recourse to cross-breeding (140).

Although methods of producing transgenic laboratory animals have been available for nearly 25 years (59), it is only recently that their potential benefit to producers, consumers and animal agriculture in general has been realised (140). A transgene is a foreign deoxyribonucleic acid (DNA) construct containing a sequence that codes for a specific protein and a promoter region that confers gene expression in specific tissues, along with insulators and other regulatory sequences to protect, enable or enhance the expression of the introduced gene (86, 110). The method predominantly used to generate transgenic livestock is microinjection of this exogenous DNA into the pronuclei of a fertilised oocyte (4, 5, 7, 141). Microinjection is inefficient owing to the random integration of the gene and the variable, often mosaic,

expression patterns in the transgenic offspring. Some of these problems have been overcome by targeting the gene for specific proteins to express in suitable organs such as the mammary gland (140, 143). The mammary gland is able to generate vast quantities of proteins, and the glandular epithelium can provide proper post-translational processing, including glycosylations (22, 45). Using this approach, various heterologous recombinant human proteins have been produced in large amounts in the milk of transgenic goats, sheep, cattle and rabbits (21, 41, 42, 45, 140, 143).

Transgenesis in swine breeding has succeeded in producing pigs that carry the gene for phytase (57). Transgenic pigs express the enzyme in their saliva and completely digest phosphorus (in the form of phytate) in their diet (57). This has encouraged breeders to look to transgenesis as a solution to the problem of manure-based environmental pollution in the pork industry (57). Other studies have shown that transgenesis may provide a means of developing lines of pigs that over-express the milk protein bovine alpha-lactalbumin and of increasing milk production in lines of low milk yield, thereby improving piglet growth rates (143). More recently, transgenic pigs that express human complement regulating proteins have been tested for their suitability as donors for human organ xenotransplants (110, 111). These studies have shown that the hyperacute rejection response can be subverted, and, in the near future, transgenic pigs may serve as donors for functional xenografts and as animal resources for a variety of xenogenic cells and tissues (110).

Cattle, which yield 10 to 20 times as much milk and protein as goats or sheep (45), would have been the favoured species for the application of transgenesis were it not for the discouraging technical problems encountered in cattle. The success rate of transgenesis in cattle is low, and the time required to assess the expression of the introduced gene is excessively long (45). These limitations can be overcome to some extent by a technique called transomatic gene transfer (22), in which DNA is injected (using a pseudotyped viral vector) into the mammary glands of a lactation-induced female. This reduces the long waiting period before the expression of the introduced foreign DNA can be tested (22). Other approaches that are currently being investigated include using spermatozoa to carry the exogenous DNA gene sequence to the zygote (23). This procedure, involving the introduction of the gene sequence into cultured spermatogonial stem cells and reintroducing these stem cells into the testis of a chemically sterilised recipient, to undergo spermatogenesis, has not yet been used successfully in cattle (23, 45). However, introducing a specific DNA sequence into the perivitelline space of bovine oocytes at the metaphase II stage, just prior to fertilisation, using a higher concentration of the vector, promises to improve the efficiency of transgenesis in cattle. Transgenesis is also reported to have been more successful following lentiviral gene transfer into bovine oocytes (45). However, one concern over the oocyte-lentiviral-vector approach is the possibility of the viral gene being incorporated into the germ line of the transgenic animal along with the gene-specific DNA.

Transgenesis in small ruminants is approximately four times more successful than in cattle, and as a result the application of transgenesis in goats and sheep has proven to be more practical. A notable example is the production of transgenic goats that secrete a spider silk protein that is stronger and more elastic than other silk fibres and is referred to as BioSteel (7, 87). The spider silk protein may be useful in the manufacture of fine (soluble) surgical sutures and artificial ligaments requiring strong or elastic fibres (87). Other proteins generated using transgenic techniques, mainly in goats and sheep, include recombinant human anti-thrombin III, alpha 1-anti-trypsin, tissue plasminogen activator, blood clotting factor IX, human butyrylcholinesterase, hepatitis antigens, and monoclonal antibodies for the production of malaria vaccine. Although some of these products are expected to become available for routine use in human medicine in due course (7, 45), it is important to note that, to date, none of these transgenically derived proteins has been approved by the regulatory bodies of any country.

Cloning in domestic animals

Another component of ART that is currently being attempted in many laboratories is nuclear transfer (NT) for producing clones. The first report on nuclear transfer in cattle appeared in 1987 when Prather *et al*. (116) produced cloned calves by electrofusing donor nuclei from two- to 32-cell-stage bovine blastomeres, with enucleated metaphase II oocytes. Over the next 13 years, over 1000 calves were produced using embryonic cells as donors. However, it was the production of lambs and calves by transferring adult cell nuclei into enucleated metaphase II stage oocytes in the late 1990s (32, 144) that spurred scientists and animal breeders to try cloning using foetal or adult somatic cells as the donors for NT, in a variety of farm animals (7, 51, 87, 146). The results of this approach in ruminants indicate the potential to produce a genetic copy (a clone) of an already proven adult animal of exceptionally high genetic merit for commercial purposes, without recourse to time-consuming progeny testing.

Cloning by somatic cell nuclear transfer (SCNT), combined with transgenesis, could play an important role in genetic improvement because of the accuracy of selection afforded by this approach and by the speed of dissemination of the introduced gene. The production of transgenic goats has been greatly improved by incorporating a DNA construct into target cells using lipid-

mediated transfection while the cells are still in culture and by selecting donor cells for NT after the proper integration of the introduced gene has been ascertained (5, 6, 7, 86, 87, 141). A good example of the potential of this approach is the transgenic cattle that carry an extra artificial mini-chromosome containing genes for human immunoglobulins. These cattle have been created by transfecting a somatic cell with a mini-chromosome carrying many genes (instead of a construct containing a single gene) to produce polyclonal human antibodies against a number of antigens, including anthrax (86).

Although nuclear transfer research conducted so far has yielded important results, the loss of reconstructed SCNT complexes is substantial in domestic animals. In cattle, a majority of SCNT clones are eliminated during blastocyst development or during the peri-implantation stages after transfer into recipients. Of those that survive gestation, a good proportion fails to be delivered at term and require induction of parturition owing to the 'large calf syndrome' (16, 45). Many of the cloned calves that are delivered die within a few days of birth owing to problems related to pregnancy and/or parturition including abnormal development and/or detachment of the placenta (45). Prolonged gestation and large offspring syndrome, accompanied by enlargement of the heart, liver and other internal organs, also occur in sheep, but not generally in cloned goats (86, 99). The 'overgrowth offspring' phenomenon, which is also seen in calves and lambs resulting from embryos produced *in vitro*, is thought to result from exposure to the *in vitro* culture system during early embryo development (99). It is believed that laboratory-derived embryos are exposed to conditions in culture that are inherently different from conditions *in vivo*, in terms of the concentrations of steroids, insulin-like growth factors (IGFs), amino acids and/or sugars (16). These unfavorable conditions are thought to lead to the breakdown of amino acids and the accumulation of harmful by-products and intra-cytoplasmic lipids, which damage the pre-placental cells and the cells of the inner cell mass, or alter the expression of imprinted genes, including IGF-2 and its receptor, that are involved in early embryonic growth and differentiation (16, 45). In addition to these culture-related disruptions to embryo and foetal growth, various facets of the SCNT process can potentially interrupt normal cell functions and harm the reconstructed embryo. These facets include cellular events related to chromatin decondensation, nuclear cytoplasmic reprogramming, cell cycle phase synchronisation, mitochondrial or nuclear DNA damage resulting from ultraviolet exposure during the procedure, chromosomal instability and aberrant gene expression. A recent study compared foetal phenotypes and gene expression in bovine foetuses and neonates resulting from *in vitro*-derived (by IVF and SCNT) embryos, with those of their counterparts resulting from embryos produced *in vivo*, using AI (75). It was found that foetuses produced by SCNT or IVF are significantly heavier and have significantly heavier internal organs than their AI counterparts. Assessment of the genome-wide 5-methylcytosine (5mC) content of the foetuses and newborns revealed that the disproportionate overgrowth phenotype is associated with hypermethylation of DNA in foetal organs, including the liver. Furthermore, a comparison of the methylation status of their internal organs with those of their respective placentas, showed that the 5mC content of placental cotyledons was lower than that of the foetal tissues indicating that the overgrowth phenomenon is linked to hypermethylation of DNA in the tissues, but not in the placentas, of foetuses derived by IVF and SCNT (75).

Regardless of the exact mechanisms involved in the elimination of blastocysts and foetuses and the abnormal growth of foetuses that survive to term, the low efficiency of current cloning techniques and the consequent high cost of producing a domestic animal by this means discourage routine application of cloning in breeding domestic animals. Even for the production of transgenic goats and sheep, the use of nuclear transfer/cloning is significantly less efficient than the pronuclear microinjection approach (7, 86, 87). However, cloned animals are reported to be exclusively transgenic and produce the recombinant (rc) proteins of interest in their milk when they are induced to lactate (86).

Currently, SCNT techniques are used mainly to generate animals for research, including animals that carry genes or chromosomes of specific interest (119). In the dairy industry, cloning is occasionally used to reproduce animals of high genetic merit, including bulls that are repeatedly ranked high on the basis of production traits but cannot produce enough semen to meet demand (18). Bousquet and Blondin (18) propose banking somatic cells from every bull entering AI facilities before they are placed on the young sire proving programme to ensure that the bulls that prove to be among the best can be cloned in the future. This approach may also prove useful in selecting bull dams, since ovarian cumulus cells (banked from the cows that produce the most milk) can potentially be used for NT (18, 45). However, NT of transgenically engineered cells may not become widespread until the techniques become more efficient and the genes responsible for economically important traits are isolated and characterised.

Gene mapping and marker-assisted selection

Various aspects of livestock production could benefit from transgenesis combined with cloning. The benefits envisaged include enhancing reproductive performance, increasing feed utilisation and growth rate, improving

carcass composition, improving milk production and composition and increasing disease resistance (105, 134, 143). However, to achieve directed genetic improvement in domestic animals, the genes controlling desirable and undesirable traits must be characterised, and this has not yet been accomplished. Techniques involving the generation of gene markers based on molecular data and the creation and use of genetic maps as selection criteria for breeding (marker-assisted selection [MAS]) may help to achieve this goal, especially in cases where pedigree data are not available or the targeted traits are of low heritability (37). The success of such techniques in breeding domestic animals has been negligible to date, especially with regard to economically important traits in cattle and other ruminants that are expressed as non-discrete phenotypes (differing in the quantities of commodities produced or in the extent of the resistance displayed to specific diseases). Although these traits have long been known to result from the additive effects of genes at different loci (78, 82, 127), the precise positions of these quantitative trait loci (QTL) on specific chromosomes and their segregation pattern during meiosis are not fully understood for any species of domestic animal. However, in the 1990s, the potential for using gene markers in progeny testing became apparent (84, 142), and studies of gene function in domestic animals were boosted by the availability of molecular tools for positional cloning (8, 37, 138). These developments made it possible to localise and sequence almost any gene or QTL using the markers (generated from microdissected chromosomes or from specific chromosomes isolated by flow sorting or from somatic cell hybrid panels) and to track the markers in the progeny and compare them with their respective phenotypes (8, 145). In this regard, information on conserved sets of genes in other mammals, including mice and humans, has been very useful in assigning groups of genes (syntenic association) to their approximate location in the respective karyotypes (48, 49, 50, 52, 69, 73, 135, 148, 149). These assignments can be further refined by using the results of studies on radiation chimeras, which help to determine the order in which these genes are located on the chromosomes of a given species (8, 48, 72, 145). Even though the linkage map, based on the respective positions of conserved sets of genes, may serve as a guide, it is the distance between two coding genes on any specific chromosome that determines the possibility of recombination (and their transmission together or separately to the progeny). This distance may vary between species and needs to be determined before the information can be used effectively in any genetic improvement programme (138).

To obtain information specific to the genes in question (exact position, nucleotide sequence, genetic distance between two genes based on physical distance and the possibility of recombination between these genes), the targeted loci must be polymorphic, with the different alleles leading to different phenotypes. In such situations, a double heterozygote (carrying contrasting alleles at two distinct loci, each with an identifiable phenotype) is created by breeding animals that display extremes of the phenotypes controlled by these loci. The meiotic segregation pattern is then assessed from the distribution of phenotypes (parental versus recombinant) among the first- and/or second-generation offspring, in relation to their marker genotype (8, 138). Since gene alteration through mutation is generally rare, coding genes are not as useful as non-coding genes as markers. The non-coding genes (repetitive sequences, including tandem repeats of mini- and microsatellites, telomeric sequences and short and long interspersed elements) constitute approximately 50% of the total DNA content (6×10^{-12} g) of a diploid bovine cell (138).

From the information gathered on the conserved genes by comparative gene mapping and by delineating microsatellite markers associated with specific phenotypes, the genes responsible for various traits in cattle and other domestic animals have been mapped to their respective loci over the past two decades. In cattle alone, some of the milk protein genes, the gene for casein kinase II, the gene for horn development, one of the genes determining coat colour (which is associated with malformation of the Müllerian duct derivatives) and the genes for mule foot, the bovine leukocyte antigens and heat shock proteins (HSP 70) were assigned to their respective loci before the beginning of this century (8, 15, 30, 31, 49, 50, 54, 73). In addition, some of the genes associated with specific disorders in cattle, including the bovine counterpart of malignant hyperthermia (which is associated with the double muscling phenotype), uridine monophosphate synthase deficiency and the neurological problem referred to as 'Weaver syndrome', have been mapped to their specific locations in the bovine genome (29, 39, 48, 53, 62, 72, 83, 108, 126). Most of these assignments concerned single gene traits, but some of these helped to track QTL associated with them. Thus, mapping of the Weaver locus led to the identification of a QTL-controlled production trait (milk production in dairy cattle) involving various genes at different loci (53, 104). Since the late 1990s, however, attention has been focused on QTL for growth rate and other production-related traits in domestic and wild animals, including American bison (103, 112, 113, 121). Information reported recently for cattle relates to such quantitative traits as growth rate, milk production, milk fat content, back fat, ovulation rate, twinning rate and various other traits related to reproduction and health (2, 36, 37, 58, 61, 85, 92, 95, 113), all of which have the potential to aid MAS in future. While substantial improvements in traits with high heritability are generally not expected from this approach, MAS is thought to be of unique value in the improvement of traits of low heritability, such as carcass traits, longevity, reproduction and disease resistance (37).

Resistance and susceptibility to specific types of disease have attracted much interest recently, and encouraging results have been obtained on the QTL and markers that control some of them. For example, it has long been known that cattle breeds differ in their susceptibility (and resistance) to the protozoan parasitic disease referred to as sleeping sickness (trypanosomosis) in certain parts of Africa (70). A recent study using an experimental cross between trypanotolerant African N'Dama cattle (*B. taurus*) and trypanosusceptible improved Kenya Boran (*B. indicus*) cattle generated valuable information on the genetic origin and possible control of this disease. Hanotte *et al.* (70) conducted an exhaustive analysis of 16 phenotypic manifestations of the disease, including anaemia, loss of body weight and parasitaemia, in 177 second-generation animals, their parents and grandparents. This study, which used 477 molecular marker QTL controlling resistance, led to the discovery of a single QTL on each of 17 bovine autosomes and two QTL on chromosome 16 (70). The individual QTL were noted to contribute between 6% and 20% of the phenotypic variance in the clinical manifestation of the trait. The authors were also able to conclude that resistance to trypanosomosis originated from the N'Dama parents, which carried 'resistant' alleles at nine QTL, while the Kenya Boran breed carried only five of these QTL (70). This study, which also detected an overdominant mode of inheritance for four of these QTL, showed that selection for high trypanotolerance within the second-generation cross could generate a synthetic breed with higher levels of trypanotolerance than the parental breeds (70).

Similar investigations of economically important traits in domestic pigs have generated a wealth of information on genes and QTL. Recently, a wild boar intercross was used to localise the QTL referred to as fluorescent antibody technique 1 to a region on the long arm of chromosome 4 that displays conserved synteny to human chromosomes 1 and 8 (111, 112). The Meishan allele for thyroxine-binding globulin (TBG) is thought to be the conserved allele found in the human, bovine, sheep and rodent TBG genes. The TBG gene is a positional candidate for testis size in boars and has been mapped, along with the QTL for body composition (backfat) and plasma follicle stimulating hormone, to an area near the centromere on the X chromosome in a Meishan-White Composite resource population. The investigators discovered a change in exon 2 of the porcine TBG gene involving a single nucleotide polymorphism (translated into a change from histidine to asparagines) in the ligand-binding domain of the mature polypeptide, and suggested that this polymorphic locus may control the variation in testicular size in boars (112).

Single genes and QTL for production traits in domestic animals continue to be mapped in the hope that these markers, along with the data on transmission patterns of phenotypes, will guide breeding programmes. Even though MAS is not commonly used to improve breeding stock at present, it will probably play a role in future genetic improvement programmes because records of relevant production traits and molecular approaches to track the genes responsible for them are being improved at a phenomenal pace.

Breeding the best and biotechnology

Recent developments in biotechnology and reproductive programmes have enabled breeders to respond readily and eagerly to the call to 'breed the best to the best, as fast as you (the breeder) can' (98). However, the increasing use of ARTs for 'genetic improvement' is likely to increase the rate of inbreeding. The most conspicuous result of close inbreeding, as stated earlier, is compromised reproductive efficiency due to the accumulation of genes that negatively affect reproduction and/or viability. In spite of this, close inbreeding (mating of brother to sister or parent to offspring) was the only way for traditional breeders to propagate desirable genetic traits. The practice of close inbreeding was not harmful in the past because it was confined to specific herds or flocks and the ill-effects could be eliminated or reduced by a generation of out-crossing (78, 98). The advent of ARTs has changed this situation drastically.

Artificial insemination, which was originally seen as a way of avoiding inbreeding, has led to mounting inbreeding, especially in dairy cattle. The international availability of semen from selected bulls has made it easy to reduce the number of males, since one or a few bulls can serve a large number of cows, both in the home country and internationally. The use of semen from different breeds and the international exchange of germplasm have led to the birth of offspring with malformations or metabolic errors that are rarely seen in natural populations (10, 13, 98, 106, 107, 108). This unwanted outcome is the result of the overuse of 'meritorious' semen, since this practice increases the number of heterozygotes in the flock and leads to the phenotypic manifestation of defective genes when daughters and granddaughters of the original donor are bred with his semen. Indeed, it is the extensive use of AI that uncovered (or confirmed) the genetic aetiology of a large number of diseases and disorders in cattle. Removal of proven carriers (sire, dam or both) from the breeding programme has reduced the frequency of some of the deleterious traits in cattle; however, their complete elimination may not be possible without reducing the size of the breeding stock, which, in turn, could lead to increased inbreeding.

Increased inbreeding also affects production-related and highly heritable traits (generally controlled by genes at different loci) and has an additive impact on the merit of

the animal (in terms of milk yield, accretion and secretion of protein and growth rate). In addition, increased inbreeding affects traits of moderate and low heritability, such as the number of offspring produced during the lifetime of the individual (98). As a general rule, inbreeding in cattle should remain below 5% over a 50-year period in a 'normal' breed (128). According to some authors, more than half the 277 clearly defined breeds of cattle found in Europe today are in danger of disappearing as a result of inbreeding (20, 128). Even in the Holstein cattle of the USA, the degree of inbreeding was approximately six-fold higher between 1980 and 1998 (0.275% per year) than between 1960 and 1980 (0.044% per year) (56). In other words, inbreeding increased only slightly in the two decades after the commercial introduction of AI in 1960, but has progressively increased owing to intense selection since 1980 (56).

Even though selection is greatly enhanced by the use of ARTs including AI, MOET and, especially, SCNT, these approaches also have the potential to increase inbreeding substantially. The possible consequences for any specific breed or population of domestic animals of using ARTs are not obvious at present since these techniques are still too inefficient to be applied routinely in breeding programmes. However, without using ARTs to generate large numbers of animals, the impact of these approaches on any genetic improvement programme will be hard to assess.

Conclusions

Biotechnology and ARTs have altered the expectations of breeders and consumers; the emphasis now is on how to achieve these expectations. Many of the techniques discussed in the foregoing sections, including oestrus synchronisation, OPU, maturation and fertilisation of oocytes *in vitro* (IVM and IVF), and culture and transfer of embryos produced *in vitro* into recipients, are already established components of ARTs. The only barrier to their routine use in animal breeding at present is their high cost relative to AI, which remains the most popular ART. Sex-sorted semen is already being used, albeit on a limited scale, in the commercial breeding of dairy cattle, in spite of the higher cost compared to AI with semen that has not been sex sorted (123, 136). Cost may be a big factor in determining whether transgenesis and cloning become routinely used in domestic animal breeding programmes; however, their acceptance may also depend upon overcoming consumer bias based on unrealistic expectations on one hand and on excessive fear of the effects of these technologies on the animals and consumers on the other hand.

The initial success with recombinant bovine somatotropin (rBST), which increases milk yield in dairy cattle, and with the creation of transgenic goats that secrete a spider silk protein of enormous strength (BioSteel) in their milk led breeders and scientists to expect much from transgenesis. The possibility of using designed gene constructs to direct an animal to generate a desired product (in its milk, urine or blood) or to grow rejection-resistant organs for use in human xenotransplants has changed approaches to testing for and treating human diseases and has polarised consumer attitudes towards genetically engineered products and ways of breeding animals. Similarly, the successful production of cloned sheep (144) and cattle (32) through SCNT fuelled expectations that the technology could be used to benefit agriculture, to replace a deceased companion animal or to repopulate an endangered or even extinct species. However, cloning and transgenesis could be used to serve less noble causes, and the perceived potential for the abuse of these technologies has engendered excessive fears of major socio-cultural changes and sparked ethical debates among scientists, politicians and the general public. The major 'genetic' concern is the potential of transgenesis and cloning to reduce genetic diversity, which is fundamental to the survival of any species. However, as discussed in the foregoing sections, the present low efficiency of cloning and transgenesis and the consequent high cost of producing an animal have prevented the wide-scale application of these techniques in domestic animal breeding programmes. Transgenesis and cloning are currently largely restricted to animals intended for research, although in some domestic animals, notably goats, these techniques are being used to generate valuable rc-proteins and to propagate transgenic founder animals that produce them (7, 86).

It is important to note that the impacts of genetically modified products and of the process of genetic modification *per se* on the animals may not be evident for a while. For example, although the beneficial effects of rBST on milk yield have been known for some time, results obtained from studies have been highly variable, and the effects on the cows treated with rBST are only beginning to be recorded. An evaluation of various parameters relating to production and nutrition, including milk production, milk composition, dry matter intake and body condition score, carried out by an expert panel established by the Canadian Veterinary Medical Association found that, while rBST increased milk production (by 11.3% in primiparous cows and by 15.6% in multiparous cows) and dry matter intake (by an average of 1.5 kg/day), the treated animals had lower body condition scores and were approximately 25% more likely to develop clinical mastitis during the treatment period (38). Cows treated with rBST were also 55% more likely to develop clinical signs of lameness and 40% more likely to fail to conceive, although the number of services needed per conception and the gestation length in cows that conceived were not affected (38). Similarly, although transgenesis has led to the successful production

of many biopharmaceuticals in the mammary glands of domestic and laboratory animals, it has also had adverse effects on animals, including premature shutdown of milk production in transgenic goats expressing human plasminogen activator in their milk (42) and leakage of the protein (erythropoietin) into the blood leading to sterility in rabbits (100). It is thought that such setbacks could be avoided by proper selection of transgene and promoter regions of the DNA construct and by pre-screening the constructs using cell lines or transgenic mice (86). However, gathering the information needed to achieve predictable and efficient results routinely, and technical refinements that reduce the cost and the effort required, are time consuming. The use of SCNT has improved the efficiency with which transgenic animals, especially goats, can be produced (5, 6, 87) since it allows the DNA construct to be incorporated into target cells in culture using lipid-mediated transfection, which in turn allows donor cells for NT to be selected based on proper integration of the gene. Although all of the animals born after using this approach are transgenic and most of them produce the rc-proteins of interest in their milk, the survival rate of NT-reconstructed embryos carrying the transgene is significantly lower than that of embryos obtained by pronuclear microinjection (7, 86). In spite of these limitations, transgenesis combined with cloning has the potential to benefit biomedicine and animal husbandry. The possibility of mass producing genetically engineered animals with desired qualities, such as transgenic goats producing BioSteel or genetically engineered sheep producing human insulin, is extremely attractive, since conventional breeding runs the risk of breeding out the desired traits through the gene reshuffling inherent to sexual reproduction.

Discussions are taking place at national and international level on the ethics of techniques such as cloning and transgenesis and vigorous arguments for and against genetic engineering and patenting of life-forms are being aired in many countries. Such discussions with consumers, producers, scientists and educators are essential to help policy-makers to develop breeding strategies that emphasise the safe, humane and ethical treatment of 'experimental' animals and to educate consumers on the real and perceived issues of product safety. These discussions could also encourage scientists to focus on developing products that are safe for human consumption without compromising the genetic improvement gained over the years and urge policy-makers to introduce regulations that guarantee caution in the use of different ARTs. However, unless genetically engineered animals and animal products are accepted by regulating bodies without excessive delay, the incentive to improve the efficiency of transgenesis and cloning and to generate more animals using these technologies may disappear along with the possibility of ever assessing the real genetic impact of these approaches on domestic animals.

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La génétique hier et aujourd'hui : sélection de l'excellence et biotechnologie

P.K. Basrur & W.A. King

Résumé

Dans le passé, les animaux domestiques étaient génétiquement améliorés par l'identification d'individus « méritants », par l'accouplement des animaux présentant les traits souhaités, par la reproduction continue d'animaux de même famille pour perpétuer leurs traits supérieurs et par croisement quand la dépression par consanguinité devenait évidente. Aujourd'hui, la reproduction assistée et la biotechnologie permettent aux éleveurs de concevoir et d'orienter l'évolution de la reproduction, de diffuser les traits souhaités et d'accélérer l'amélioration génétique. L'intervalle intergénérationnel peut être sensiblement réduit grâce à la combinaison de l'insémination artificielle, qui est la technologie de reproduction assistée la plus ancienne et la plus utilisée, avec des méthodes

plus récentes, notamment la synchronisation œstrale, la superovulation, la récolte d'ovules chez des femelles n'ayant pas atteint leur maturité sexuelle, même en dehors de la saison de reproduction, ainsi que la production d'embryons *in vitro* et le transfert d'embryons. De plus, les caractéristiques sexuelles et génétiques de la descendance peuvent désormais être sélectionnées grâce à la détermination du sexe du sperme pour l'insémination, la sélection assistée par marqueurs, la suppression ou l'ajout fonctionnels de gènes spécifiques dans le génome de la descendance, et le clonage par transfert nucléaire de cellules somatiques. Toutefois, le faible taux de réussite de certaines de ces méthodes a retardé leur application à grande échelle, ce qui a entravé l'évaluation de leur impact génétique. Les conséquences génétiques potentielles de certaines de ces méthodes doivent être évaluées aussi soigneusement que les procédures destinées à surmonter leurs inefficiences « techniques ».

Mots-clés
Amélioration génétique – Consanguinité – Sélection assistée par marqueurs – Techniques de reproduction assistée – Transgenèse.

La genética ayer y hoy: selección de los mejores y biotecnología

P.K. Basrur & W.A. King

Resumen
En el pasado se acostumbraba a mejorar la dotación genética de los animales domésticos seleccionando a los ejemplares con características deseables, apareándolos entre sí y con su descendencia para perpetuar los rasgos superiores y recurriendo al mestizaje cuando se advertían signos de depresión por consanguinidad. Hoy en día, gracias a la reproducción asistida y la biotecnología, los criadores planifican y dirigen el proceso de reproducción, diseminan los rasgos deseados y aceleran la mejora genética. El intervalo intergeneracional puede ser acortado sensiblemente, gracias a la combinación de la inseminación artificial, que es la más antigua y utilizada de las técnicas de reproducción asistida, con las técnicas más recientes que son el estro sincronizado, la superovulación, la extracción de óvulos de hembras sexualmente inmaduras, aun fuera de la época de cría, así como la producción de embriones *in vitro* y la transferencia de embriones. Además, es posible seleccionar el sexo y la configuración genética de la progenie mediante o con semen sexado para la inseminación, la selección mediante marcadores, la deleción o adición funcionales de genes específicos en el genoma de la progenie, o la clonación por transferencia de núcleos de células somáticas. Sin embargo, el hecho de que algunas de estas técnicas deparen un índice de éxitos bastante bajo ha retardado su aplicación a gran escala, lo que a su vez dificulta la correcta evaluación de sus efectos genéticos. Sería preciso evaluar cuidadosamente tanto las consecuencias genéticas de algunas de estas aplicaciones como los procedimientos para subsanar sus deficiencias 'técnicas'.

Palabras clave
Consanguinidad – Mejora genética – Selección mediante marcadores – Técnica de reproducción asistida – Transgénesis.

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