April 1999

COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

RECENT DEVELOPMENTS IN BIOTECHNO LOGY AS THEY RELATE TO ANIMAL GENETIC RESOURCES FOR FOOD AND AGRICULTURE

by E.P. Cunningham

The Commission has regularly received reports on new developments in biotechnology, in relation to plant genetic resources for food and agriculture. Document CGRFA-8/99/Inf.9 provides information to the Commission on this rapidly developing area.

With the broadening of the Commission to include animal genetic resources for food and agriculture, the present document provides complementary information, specific to animal genetic resources for food and agriculture. The study is the responsibility of the author, and does not necessarily represent the views of the FAO, or its Member States.

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For reasons of economy, the paper is available only in the language in which it was prepared.

**RECENT DEVELOPMENTS IN BIOTECHNOLOGY AS THEY RELATE TO
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1.   Introduction

Livestock form the basis of farming systems in varying degree throughout the world. Two thirds of the world's farmed land, for example, supports only pastoral systems. Throughout the developed countries, the livestock sector accounts for over half of agricultural output. Even in crop dominant systems, as in South East Asia, livestock continue to be an essential component of the system. Throughout the developing world, production in the livestock sector is increasing 50% more rapidly than in the foodcrop sector. As incomes increase, and consumption patterns change, this increasing place of livestock in agricultural systems is expected to continue[2].

With finite resources and an increasingly vulnerable environment, it is critically important that growth in efficiency rather than in numbers should be the dominant factor in the doubling of global output of livestock products expected to take place in the next 25 years. Increasing efficiency of production is also essential for the economic and physical sustainability of many different farming systems, as well as being the key to long term reduction in the cost of food.

Improvements in efficiency arise from the development, spread and adoption of improved technologies for breeding, feeding, management, and healthcare of animals. In these respects the definition of efficiency must be production system specific. Additionally, improved technologies are required for protection of animal welfare, conservation of genetic resources, management of livestock-environment interactions, efficiency of processing and marketing of livestock products, and to enhance the nutritional and consumer safety aspects of livestock derived foods.

As the benefits of improved technology are harvested in evolving livestock systems in many developing countries, new pressures are brought to bear on the animal populations on which these systems are built. Often, the pressure is for replacement of local strains. In other cases, it involves the dilution of local gene pools by cross breeding. The development of world agriculture has produced almost 5,000 separately identified breeds and strains of the main farmed livestock species. In many cases, these strains represent the result of many hundreds or even thousands of years of adaptation to local conditions. As the pace of change quickens, it is necessary to ensure these benefits are properly evaluated, that realistic opportunities are provided for the continued use and development of local strains as appropriate, and where desirable local strains are properly conserved for the future. Some of the new technologies can assist in these tasks.

The primary objective of this position paper is to review the main reproductive and genetic technologies currently under development for the livestock sector; to evaluate their potential impact on the utilisation of animal genetic diversity and on their conservation, for the sustainable intensification of production systems.

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[1]   Professor of Animal Genetics, Trinity College Dublin, Ireland. Contributions by the various experts who read drafts of the analysis are greaty acknowledged .

[2]   FAO (1996). World Agriculture: Towards 2010, (Ed. Alexandratos, N.) FAO / Wiley.

2. Reproductive Technologies

2.1 Artificial Insemination

Artificial Insemination (AI) has now been a practical technology in cattle for over fifty years. The benefits of AI, originally primarily developed to overcome venereal diseases, but now linked to intensive male selection have led to the virtual replacement of natural service with artificial insemination throughout the commercial dairy populations of particularly the developed world. In commercial beef cow populations, oestrus detection is more difficult and use of AI less convenient. Bull progeny testing offers less advantage than in the dairy case. These factors combine to give AI usage rates generally below 10% in beef cattle.

In many developing countries, the combination of factors needed to make widespread AI usage economic are not yet present. Output value per cow is low, so AI costs a high proportion of output. Nutritional stress on cows, together with less efficient transport and communication, make AI technically less efficient. There are seldom sufficient cows in milk recording for effective progeny testing and many such populations are involved in cross breeding and source their semen externally.

The extent of AI use in cattle has been surveyed separately in developed[3] and developing[4] countries. The results showed a world-wide total of some 50 million first inseminations per year. The developed economies, with approximately 30% of the world's cattle, were responsible for 83% of the global AI activity. In these countries, total AI numbers are declining in line with numbers of dairy cows. AI in some developing countries, on the other hand, is increasing rapidly, particularly in Asia, though from a low base of close to 5 million first AI. Given that AI is a mature and established technology for some farmed species, its future development will be largely driven by economic factors. In developing countries, there is undoubtedly great scope for expanded use in breed development, and this is likely to take place mainly in the context of development of more intensive periurban dairying systems.

Now in developed countries AI is also important in the breed development of other species such as sheep, horses and turkeys and increasingly in the development of pig genetic resources.

Long term semen storage, without loss of viability, is a valuable technique for assisting conservation in endangered breeds of most of the more important farm animal species. It has the disadvantages of preserving only half genotypes and requiring secure cryo-preservation facilities. However, these are balanced by its long term fertilizing reliability and low cost. In parallel with *in vivo* conservation, it is the first technology which should be considered.

Policy issues primarily concern the purposes for which AI is to be used. In the past, many mistakes have been made by introducing the technique where it was economically unsustainable, and where the net effect was a transient injection of foreign genes into the local population. On the other hand, where well-structured dairy development programmes are in place, with cattle or buffalo, it can play a valuable role in facilitating appropriate genetic change in the population. This has been well demonstrated, particularly in India. The location and establishment of conservation banks to enable further characterisation, sustainably maintain endangered breeds and manage access to unique genetic resources is also likely to involve a number of policy issues nationally and regionally.

2.2 Embryo Transfer

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[3]   Chupin, D. & Thibier, M. 1995. Survey of the present status of the use of artificial insemination in developed countries. *World Animal Review* 82: 58-68.

[4]   Chupin, D. & Schuh, H. 1993. Survey of present status of the use of artificial insemination in developing countries. *World Animal Review* 74 / 75: 26-35.

Over the past twenty years, methods of recovering, storing, and implanting cattle embryos have been advanced. It is now possible to recover non-surgically up to thirty embryos at a time, though average yield is about 5.5, and high between-animal variability in embryo yield remains a problem.[5] Freezing in liquid nitrogen and thawing have relatively small affects on viability.

The chief benefit of increasing reproductive rates of selected cows is that genetically outstanding cows can contribute more to the breeding program. The term MOET (Multiple Ovulation Embryo Transfer)[6] describes a closed system in which these techniques are used to increase average rates of genetic gain. MOET type schemes have been initiated in at least 8 countries, though in all cases in open or hybrid form. With these modified MOET schemes increases of about 10% in expected rate of genetic gain are possible.[7]

In principle, MOET type nucleus breeding schemes should have significant advantages in developing countries where the absence of on-farm recording makes conventional progeny testing impossible. This is particularly true for milking buffalo populations which do not have the option, available in cattle, of cross-breeding with highly selected breeds. The Indian National Dairy Development Board has been involved since 1986 in developing such an ET based scheme for buffalo. To date, about 1,000 embryo transfers have been carried out in this program. Nevertheless, MOET schemes do involve a high level of organisation and management to be effective, both centrally and in the necessary wide dissemination of the realized improvement over time to the farming community.

Embryo transfer can also be used for some specialised purposes such as rapid expansion of rare genetic stocks, reducing the cost of international transport by shipping embryos rather than live animals, the rapid replacement of existing genotypes by using ET rather than grading up through continuous crossing, and the possibility of increasing the twinning rate by following AI with a transplanted embryo.

Because the cost of ET is high, use is almost confined to the trade in high value pedigree animals. In contrast to AI, total reported bovine ET numbers are increasing, and approximately 460,000 embryos were transferred world-wide in 1997.[8]

Policy issues concerning embryos are analogous to those for AI. In developed countries, it is a commercial business, and there has been some concern that the technique might accentuate the depletion of genetic variation within populations. However, at current and prospective usage levels, this effect will be marginal. In developing countries, its use will be quite restricted for economic reasons, and its main value will be in nucleus breeding schemes and in supporting conservation programmes. These applications will necessarily be driven by public rather than private sector interests.

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[5]   Thibier, M. 1998. The 1997 statistics on the world embryo transfer industry. *Embryo Transfer Newsletter* Vol. 16 : No. 4: 17-20

[6]   Nicholas F.W., & Smith C. 1983. Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Anim. Prod.* 36: 341-353.

[7]   Lohius, M.M. 1995. Potential benefits of bovine embryo-manipulation technologies to genetic improvement programs. *Theriogenology* 43: 51-60.

[8]   Thibier, M. & Nibart, M. 1995. The Sexing of Bovine Embryos in the Field. *Theriogenology* 43: 71-80..

2.3   Embryo Cryopreservation

Freezing of semen and embryos, particularly in cattle, is well established commercial practice. Its use has also been proposed for the conservation of endangered species and breeds. In that context, embryo conservation should be substantially more useful than that of semen, since complete genotypes can in this way be conserved.

Since the first bovine pregnancy from a frozen embryo 25 years ago, more than 800 experimental reports and papers have been published on various aspects of embryo cryobiology.[9] Recent developments in vitrification where high molecular weight cryoprotecive agents are being used allowing freezing without costly programmable freezing units. Long-term storage is normally in liquid nitrogen. The critical stages are the freezing and thawing, and much research has been devoted to developing effective methods of preserving viability through these stages. Freezing is done in the presence of a cryoprotective additive which prevents crystallisation of cell water. Thawing to physiological temperatures was originally done in careful stages, but new cryoprotectants have been developed which permit quick thawing of embryos. This makes the transfer procedure very much analogous to AI.

Frozen embryos show consistently lower fertility, by about 10%, than those transferred fresh. Freezing and thawing superimposed on IVF incur further penalties in net fertility. The effects of long-term storage cannot, by definition, be evaluated in the short-term. However, if the analogy to long-term semen storage is taken as a guide, then no additional losses should occur over a period of several decades at least. These assumptions lead to the conclusion that somewhere between a quarter and a half of long-term frozen bovine embryos could result in a living animal. As a conservation strategy for an endangered breed, this should be a worthwhile option, since the embryos selected for freezing can be chosen to represent the maximum range of current diversity.

Because of the great amount of work on cattle embryos, the prospects for using cryopreservation for conservation purposes are greatest in that species. Similar possibilities should be within reach in sheep. In pigs, many difficulties are present, and success rates are low.[10] In this and other farm animal species considerably more work is required before long-term embryo cryopreservation can be considered a reliable conservation technique.

2.4   IVF Embryo Production

Research in recent years has concentrated on a new package of technologies. Immature eggs (oocytes) are collected either from living animals or from the ovaries of recently slaughtered animals. These are then matured and fertilised in the laboratory, and cultured on to a certain stage, at which point they are either transferred to a recipient animal or frozen for later transfer. This process is known as IVM/IVF (*in vitro* maturation, *in vitro* fertilisation), or simply IVF.[11]

The process of oocyte recovery was first concentrated on the use of slaughterhouse ovaries. This is now largely replaced by a method for aspirating immature oocytes (Ovum Pick-Up) from the ovaries of living cows.[12] This means that high quality animals can be accessed, and experience has

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[9]   Leibo, S.P. & Loskutoff, N.M. 1993. Cryobiology of *in vitro-* derived bovine embryos. *Theriogenology* 39: 81-94.

[10]   Nagashima, H., Kashiwazaki, N. Ashman, R., Grupen, C., Seamark, R.F. & Nottle, M. 1994. Recent advances in cryopreservation of porcine embryos. *Theriogenology* 41 : 113-118.

[11]   Tervit, H.R. 1997. *In vitro* production of cattle embryos. In : Milk Composition, Production and Biotechnology 341-355. (Eds. Welch, R.A.S. *et al.*) CAB International, Wallingford.

[12]   Pieterse, M.C., Kappen, K.A., Kruip, Th.A.M.& Taverne, M.A.M. 1988. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. *Theriogenology* 30: 751-762.

shown they can safely be aspirated repeatedly, even while pregnant. When high merit donor cows are linked to a good IVF laboratory, this technique could be used to produce high quality embryos in quantity. Early research results suggest that freezing of bovine oocytes will be possible but considerable research remains to develop a reliable and practical technology. Freezing of oocytes in combination with frozen semen would provide important future mating regime flexibility in the conservation programme.

For all IVF technologies, achieving economically viable success rates is still a problem. Less than 30% of cultured oocytes develop into transferable embryos. Wastage during pregnancy is also increased, with IVF embryos achieving about 10% lower success rates than conventional embryos, where pregnancy rates of about 50% are regularly achieved.[13] Freezing of embryos causes a further slight drop. Research is focused on removing this penalty, as well as on improving the efficiency of oocyte recovery, IVM, IVF and embryo survival. Despite these difficulties, IVF laboratories are functioning in a number of countries.

2.5   Sexing Semen and Embryos

If the sex of an embryo could be determined in advance, this should be an advantage to the end user, and hence add to the value of the product. With present technology, it is possible to extract one cell from an early embryo, and with the use of a DNA probe to know whether it is male or female.[14] However, this technology has not been widely used because of high cost and reduction in fertility.

The sex of an embryo is determined by whether the fertilising sperm had an X or a Y chromosome. Much research has been devoted to attempts to separate X from Y bearing sperm. Methods based on sedimentation, centrifugation, electrophoresis, and surface antigens have all proved ineffective. A newer approach, initially developed by the United States Department of Agriculture, offers the prospect of radically improving the prospects of gender control.[15] This technology involves sorting the semen, one sperm at time, into male and female. Because male and female sperm carry different sex chromosomes, they differ slightly (by 2.8% in the case of cattle) in the amount of DNA they contain. When they are properly stained, this difference can be detected by a laser beam and the sperm can be sorted using standard flow cytometry equipment.

This system functions well, but still with some serious limitations. Sorting is about 90% correct and fertility is reduced. With current equipment about one thousand sperm per second can be sorted, too slow for mass pre-sexing of semen for normal AI use. In an attempt to reduce the number of sperm required, low sperm count insemination can be used, though with reduced conception rates.[16] The prospect of commercial use is still some years away.

In an IVF context, only a few thousand sperm are needed for fertilisation. Indeed, single sperm injection has been shown to work. With existing success rates, it would be possible to use sex-sorted semen for IVF embryo production on a substantial scale. Costs should be reasonably low, and almost all embryos produced would be of whichever sex is desired. Problems which remain to

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[13]   Hasler, J.F. *et al*. 1995. Production, freezing and transfer of bovine IVF embryos and subsequent calving results. *Theriogenology* 43: 141-152.

[14]   Thibier, M. & Nibart, M. 1995. The Sexing of Bovine embryos in the Field. *Theriogenology* 43 : 71-80.

[15]   Johnson, L.A., Cran, D.G., Welch, G.R. & Polge, C. 1996. Gender pre-selection in mammals. Proc. XX Beltsville Symposium: 'Biotechnology's Role in Genetic Improvement of Farm Animals. 151-165. (Eds. Miller, R.H. *et al*.) USDA.

[16]   Seidel, G.E., Allen, C.H., Johnson, L.A., Holland, M.D., Brink, Z., Welch, G.R., Graham, J.K. & Cattell, M.B. 1997. Uterine horn insemination of heifers with very low numbers of nonfrozen and sexed spermatozoa. *Theriogenology* . In Press.

be overcome include the cost, genetic quality and availability of oocytes; culture regimes for consistent success in IVM / IVF; and attaining acceptable fertility rates with frozen semen which is thawed and then sorted, and with fresh semen which is sorted and then frozen. Commercially viable systems should be possible, but are unlikely to be available in the near future.

2.6   Cloning

The first successful cloning in vertebrate animals was reported in 1952, in frogs. Cloning of domestic animals was first achieved in sheep over ten years ago.[17] At that time, cloning research was concentrated on early embryonic source material, since it was believed that beyond that stage irreversible differentiation of cells had taken place. Identical individuals were first produced by physical splitting of embryos. It was thought that repeated culture and splitting could produce large numbers of identical individuals, but this has not proved possible.

An alternative technique, based on nuclear transplantation has proved more successful.[18] Nuclei from cells of early embryos are transferred to unfertilised eggs from which the nucleus has been removed. However, embryo survival (beyond 55 days in the bovine) has been rare, and the prospect of repeated cycles of cloning and embryo culture being simple to achieve has not been fulfilled.

Following earlier success with an embryonic cell line,[19] a major advance was achieved in 1997[20] demonstrating, for the first time, the possibility of cloning from body or somatic cells of adult animals. Existing nuclear transfer techniques were used, but with nuclei from cell lines derived from mammary tissue of an adult sheep. Subsequently, fertile cloned mice were produced from cumulus cells collected from metaphase II oocytes.[21] More recently, eight cloned calves were produced from cumulus and oviduct epithelial cells of one adult cow.[22] In each cow, the culture of the donor cells had been designed to reprogram the cells to their pre-differentiation state. The key step appears to be the induction of a period of quiescence by serum starvation of the cultured cells. Note that the majority of genes in the cells of a particular adult tissue are inactive, for they are not involved in maintaining the particular body processes for which that tissue or organ is required. For somatic cloning to be realized this DNA quiescence had to be reversed. These developments will undoubtedly stimulate a great increase in the volume of research on cloning. This may increase success rates and reduce costs to the user.

An advantage of cloning somatic over embryonic tissue is that the former provides the opportunity to first observe the merits of the animals to provide the cells used for cloning. Somatic cloning also offers the possibility of sampling readily accessible and robust cellular tissues for conservation and more cost-effective research by utilising as experimental animals numbers of clones obtained from divergent donor animals. A small group of experts supported by FAO and the Government of Italy has evaluated the opportunities and needs for the use of somatic cloning in the conservation of animal genetic resources at risk, making a number of firm recommendations for action, particularly

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[17]   Willadsen, S.M. 1986. Nuclear transplantation in sheep. *Nature* 320 : 63-65.

[18]   Wolfe, B.A. & Kraemer, D.C. 1992. Methods in bovine nuclear transfer. *Theriogenology* 37 : 5-15.

[19]   Campbell, K.H.S., McWhir, J., Ritchie, W.A., & Wilmut, I., 1996. Sheep cloned by nuclear transfer from a cultured cell line. *Nature* 380: 64-66.

[20]   Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. & Campbell, K.H.S. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature* 385 : 810-813.

[21]   Wakayama, T., Perry, A. C. F., Zuccotti, M., Johnson, K. R. & Yanagimachi, R. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature. 394: 369.

[22]   Kato, Y., Tani, T., Sotomaru, Y., Kurokawa, K., Kato, J., Doguchi, H., Yasue, H. & Tsunoda, Y. 1998. Eight calves cloned from somatic cells of a single adult. *Science* 282: 2095-2098.

for the development of protocols for field use in sampling, preliminary storage and transport of cell tissue types to enable low cost sampling for conservation purposes of animal genetic resources at risk in remote developing country areas where sampling and storage of adequate samples of semen and embryos is not practical.[23]

A widely reported problem with animals derived from nuclear transfer embryos concerns abnormalities of general and specific tissue growth both in utero and after birth. The experience included extended gestations, increased still-birth rates and perinatal deaths.[24] It is not known whether these abnormalities are the result of the nuclear transfer procedure, or of elements of the IVM/IVF culture process. Further research will be required to identify the source of these abnormalities, because, even at low frequency, their occurrence is sufficient to inhibit uptake of any technology involving embryo culture.

Cloned embryos, at least in cattle, could be a competitive technology for some livestock genetic improvement purposes. In organised dairy cattle breeding programs linked to AI, annual genetic gain for an index of production traits of 1% to 2% is now being regularly achieved. With efficient cloning, and efficient testing as a basis for selection among clones, the rate of gain could, in principle, be increased. A number of studies have concluded however, that, the potential gains are unlikely to be sufficient for cloning to be justified in accelerating genetic improvement in dairy cattle breeding.[25] [26]

Genetic gain produced in large organised breeding schemes is at present disseminated mainly through AI, with a small impact from the use of conventional embryos. With the breakthrough on cloning from adult cells, the reprogramming of cell lines derived from adult tissue could open the way to large scale clonal propagation of outstanding individuals. It remains to be seen whether technical success rates will be sufficient to provide such clones at reasonable cost. However, there can be little doubt that there would be strong commercial demand and that this development could therefore become very significant in dairy cattle breeding.

Because of the low success rates and high costs in achieving a functional transgenic animal, there could be great commercial interest in multiplying such animals. Unless this situation can be overcome, transgenics are likely to be of primary interest in the pharmaceutical industry rather than in food production (see section 3.3).

In crossbreeding between developed B*os taurus* dairy breeds and B*os indicus* local breeds in tropical countries, considerable heterosis (of the order of 25%) has been regularly observed in the first cross (F1).[27] This is relatively easily achieved in the first generation by using semen of the B*os taurus* dairy breed. However, no subsequent breeding strategy retains the full amount of heterosis. Strategies which attempt to maximise heterosis retention are generally too complex for implementation. Embryos could be produced by IVF, using B*os indicus* semen on B*os taurus*

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[23]   FAO. 1998. The Implications of reversible DNA quiescence and somatic cloning for the conservation of animal genetic resources. In: Resources > Library > Reports of Meetings. URL http://www.fao.org/dad-is/

[24]   Kruip, Th.A.M. & den Dass, J.H.G. 1996. *In vitro* produced and cloned embryos : effects on pregnancy, parturition and offspring. *Theriogenology* 47 : 43-52.

[25]   de Boer, I.J.M., Meuwissen, T.H.E., & Van Arendonk, J.A.M., 1994. Combining the genetic and clonal responses in a closed dairy cattle nucleus scheme. *Anim. Prod*. 59: 345-358. Ruane, J., Gunnar, K. & Sehested, E. 1997. Views on the potential impact of cloning on animal breeding and production. Acta Agric. Scand., Sect. A, *Animal Sci*. 47 : 209-212.

[26]   Ruane, J., Gunnar, K. & Sehested, E. 1997. Views on the potential impact of cloning on animal breeding and production. Acta Agric. Scand., Sect. A, *Animal Sci*. 47 : 209-212.

[27]   Cunningham, E.P. & Syrstad, O. 1987. Crossbreeding *bos indicus* and *bos taurus* for milk production in the tropics. FAO Animal Production & Health Paper 68.

oocytes collected either *in vivo* or at a slaughter plant. Alternatively, it might in the future be possible to use embryos cloned from cells of outstanding F1 individuals.

The economics of this application of this technology have been examined.[28] It is likely to be of interest primarily in the humid tropics, where the heterosis displayed by F1 individuals has greatest value. It will also require reasonable profitability per cow, and good support services.

Another example of cloning is the primordial germ cell technique[29] being developed in poultry for producing germ line chimeras. It has potential use for preserving the foundation stocks in the case of unexpected diseases or other unexpected events, and to augment a poultry genetic resource which is endangered under natural mating conditions, and also for producing transgenic poultry.

The full policy issues surrounding cloning of farm animals are not yet clear, and depend on the scale of use. The first convincing evidence that cloning from adult cells might be practicable on a widespread scale was published as recently as December 1998. Assuming that these developments lead to reliable adult cloning at relatively low cost, then the policy implications will be very considerable. If the cost of high quality cloned embryos could be brought down to within a few multiples of the cost of AI (say under $100), then widespread application could be expected in developed dairy populations, with further impact on genetic variation already reduced by the use of AI. However, in a commercial world this is likely to be self correcting, since producers would wish to counteract the negative effects of reduced variation by seeking embryos with increased levels of heterozygocity. Technology could respond to this by using outstanding crossbred animals as the source of the clonal material.

In some developing country production systems real technical benefits could be possible through the provision of cloned female hybrid embryos for milk production. If technical reliability can be brought to an acceptable level, and costs similarly be brought within acceptable limits, this technology has the potential to provide both an economically and genetically sustainable basis for milk production in the long term. Exploring, and eventually exploiting, this potential should be a high priority.

3.   Genetic Technologies

3.1   Genome Maps

Genetic differences between individuals are due to the different genes that they carry. A gene is a stretch of DNA which codes for a particular protein. It is estimated that each individual animal has up to a hundred thousand such genes.
Some of these genes have simple and direct effects, such as coding for coat colour. This means that individuals carrying them can be easily identified and selected. However, for animals in particular that situation is exceptional. For most traits, including economic ones like milk or meat production, many genes interact to produce the end effect, and it is difficult to identify any single gene that has a significant part to play. To accurately identify individuals with superior genes, it is therefore often necessary to spend a great deal of time and effort to measure the performance of large

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[28]   Jarvis, Lovell S. 1996. The Potential Effect of Two New Biotechnologies on the World Dairy Industry. Westview Press. Boulder, Colorado.

[29]   Simkiss,K. 1997. Embryo Manipulation of the germplasm, *Poultry Science*,

          76:1093-1100.

numbers of their relations (including progeny), and to compare the results carefully with similar data for other individuals.

Within the past ten years, advances in DNA technology offer the prospect of identifying genetically superior individuals in a much more direct way. The functional genes in each individual constitute only a small fraction (under 5%) of that individual's total DNA. Most of the rest has no known function. However, scattered throughout are thousands of small pieces of DNA, called *microsatellites*, which have turned out to be remarkably useful. Each microsatellite contains a number (typically 5 to 15) repeats of very short DNA sequences. They can be amplified using the *polymerase chain reaction* (PCR). If a particular microsatellite is located close to a useful functional gene (say a gene for higher milk protein synthesis), then they will tend to be inherited together. The microsatellite can then be used as a marker for the functional gene.

The first stage in using these microsatellites in this way is to construct a marker (or linkage) map covering the whole genome. This is well advanced in human, mice and increasingly in farm animal species. The most recent summary[30] records the total number of mapped loci for cattle at 2850, 1774 for pigs and over 1000 for sheep. The highest resolution single linkage maps in each species contained 1425 markers for cattle, 1250 for pigs and 500 for sheep. The goal is to produce sufficiently dense genetic maps to assist in the search for a) single gene traits of economic importance, and b) quantitative trait loci (QTL) which contribute substantially to the continuous variation observed for most genetic traits of economic importance. These linkage maps will be used to develop strategies for marker-assisted selection (MAS) aimed at achieving more rapid genetic improvement of the traits of importance.

3.2   Marker Assisted Selection

The use of genetic technology and MAS in animal production has moved from a theoretical concept to the beginnings of practical application during the 1990s. The low and medium density linkage maps that have been constructed generally consist of several hundred to over a thousand microsatellite markers distributed throughout the genome. Using well established statistical techniques, and specially constructed three generation families, linkage between these markers and production traits can now be established.

While this provides encouragement that there are potentially useful genes to be found, it is of little immediate value in selection, because the regions of the genome involved tend to be large. Further cycles of work are therefore required to locate the gene or genes involved more precisely. The methodology for creating these higher density maps is still evolving and a number of different approaches are being used.[31]

- Positional cloning of markers from defined regions by chromosome micro-dissection, or use of yeast (YAC) or bacterial (BAC) artificial chromosomes.

- By cross-reference to the much better documented human and mouse genomes, it may be possible to infer the approximate location of a functional gene, and also something about the nature of its action.[32]

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[30]   Kappes, S.M. 1999. Utilization of Gene Mapping Information in Livestock Animals. *Theriogenology* 51: 135-147.

[31]   Georges, M. & Andersson, L.1996. Livestock genomics comes of age. *Genome Research* 6 (10) : 907-921.

[32]   Womack, J.E. & Kata, S.R. 1995. Bovine genome mapping : evolutionary inference and the power of comparative genomics. *Current Opinions in Genetics & Dev.* 5 : 725-733.

- In populations where a trait has been subject to recent and effective selection, blocks of DNA around a favourable gene may have been less disrupted by recombination than similar sized blocks in non-selected regions. By studying the variability around such regions, and how it differs between selected and non-selected populations, it may be possible to infer the location of important genes.[33]

In the shorter term, increasingly dense linkage maps of animal genomes should facilitate practical applications of MAS for single gene traits and QTL, ranging from improvement of simple production characteristics to more elusive traits such as disease resistance or product quality factors. Furthermore, parallel work in laboratory species may speed the task. Current research on trypanosomiasis provides a good example. Infection with the blood parasites *Trypanosoma congolense* and *T. vivax* is the most important animal disease in Africa, with direct and indirect losses estimated at more than $5 thousand million per year. Conventional controls (vaccines, chemotherapy, vector suppression) are unavailable, expensive or difficult to implement effectively. There is therefore great interest in understanding and exploiting the inherited trypanotolerance of some West African breeds. Locating genes involved would be of great significance, but the necessary work in cattle is very expensive and takes many years. QTL with significant effects for trypanotolerance in mice, have been identified on chromosomes 5 and 17.[34] A preliminary analysis of parallel work in cattle suggests the presence of a trypanotolerance locus on bovine chromosome 23.[35] Likewise, it may prove possible to control such devastating and difficult diseases as African Swine Fever by similar means. There are field observations which strongly suggest that resistance may be inherited but clearly the necessary rigorous research must be done.[36]

Clearly, the potential genetic benefits in livestock production and health from the developments in molecular biology are very great. There are, however, also factors which moderate this expectation. Several theoretical studies have attempted to measure the possible contribution of marker-assisted selection to increased rates of genetic gain, particularly for performance traits measurable on most animals in dairy cattle. In general, the calculated increases are modest - even with favourable assumptions being of the order of 20%. The sustainability of such gains over a number of generations is also questioned, as effective marker-assisted selection could lead to reduction in genetic variance, reducing the scope for selection gains in the future. A further moderating factor may be the cost of conducting DNA characterisation on a widespread scale, though costs are likely to come down as new technologies are developed for this purpose.

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[33]   Charlier, C., Farnir, F., Berzi, P., Vanmanshoven, P., Brouwers, B., Vromans, H., & Georges, M. 1996. Identity-by-descent Mapping Traits in Livestock : Application to Map the Bovine Syndactyly Locus to Chromosome 15. *Genome Research* 6 :580-589.

[34]   Kemp, S.J., Iraqi, F., Darvasi, A., Soller, M. & Teale, A.J. 1997. Localization of genes controlling resistance to trypanosomiasis in mice. *Nat .Genet* . 16(2) : 194-196.

[35]   Teale, A., Iraqi, F., Darvasi, A. Hanotte, O., Gathuo, H., Sileghem, M., Womack, J.E., Soller, M. & Kemp, S. 1996. Genetics of resistance to trypanosomiasis in mice and livestock. *Animal Genetics* 27 (Suppl. 2): 1-16.

[36]   FAO-IAEA. 1999. The feasibility of the genetic control of African Swine Fever. Draft Report of a Workshop.

3.3   Transgenesis

Gene transfer (or transgenesis) means the stable incorporation of a gene from another species in such a way that it functions in the receiving species and is passed on from one generation to the next. In mammalian species, the transfer is mostly done by direct injection of the foreign DNA into the nucleus at the early embryonic stage. Gene transfer has been achieved in all the major livestock species, and since the first success in 1985, more than 50 different transgenes have been inserted into farm animals. Because so many separate steps are involved, the success rates are often low - usually one or two per cent. This imposes an enormous cost in the case of cattle; so most work has been done in mice, pigs and sheep.

As a broad generalisation, it can be said that in farm animal species about one in ten injected and transferred embryos survives, and about one in ten of these carries the transgene, or transferred genetic construct.[37] Among these transgenic animals, further wastage can be expected. Normally about half express the transgene. In those, which do show expression, the gene may be activated in unintended tissues or at abnormal times in the animal's development. This unpredictability of gene expression is perhaps not surprising, given that it is currently not possible to control the site of integration into the host genome, nor the number of copies integrated. Furthermore, transgene transmission to the next generation is sometimes abnormal. The expectation would be that 50 % of offspring would inherit the transgene. However, this is true in only 70 % of the cases in mouse studies. The most widely accepted explanation is mosaicism, where the transgene is present only in some cells of the developing parental embryo. It is clear that a great deal more detailed research will be required to overcome these difficulties. A particularly promising approach is to develop methods of verifying that an injected embryo actually carries the transgene before it is implanted. In principle, this could increase success rates tenfold.

It is difficult to see production of transgenic livestock contributing to improved efficiency of animal production until the efficiency of the process of producing transgenics is dramatically improved. Predictability of the timing and location of expression will also need to be improved. One consequence of variable expression has been to produce unacceptable side effects on the health and welfare of animals. Consumer concern from lack of convincing information on transgenics and antipathy to transgenesis is very strong in many countries, and both producers and consumers would reject a technology which had negative effects on animal welfare.

Furthermore, transgenesis for enhancing livestock production must compete with other technologies. Genes promoting productivity (meat, milk, wool) or reducing costs (disease resistance) are most likely to be found within the species concerned. Normal selection programs can be quite efficient in utilising them where there measurement and recording in herds/flocks is feasible. The use of markers could add to the efficiency of the process. If a gene is sufficiently well characterised to permit its use in transgenesis, then it will also be possible to genetically characterise individuals carrying the gene, and to make direct selection and propagation highly efficient.

The objective of gene transfer is to produce in the animal a protein which it does not normally produce. This can be done for two kinds of proteins. The first group would be expected to improve the normal functioning of the animal. In dairy animals, most consideration has been given to genes which modify fat or protein synthesis in the mammary gland.[38] There is a possibility that the

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[37]   Wall, R.J. 1996. Modification of milk composition in transgenic animals. Proc. XX Beltsville Symposium : Biotechnology's role in Genetic Improvement of Farm Animals. 165-189. (Eds. Miller, R.H., *et al*.) USDA.

[38]   Vilotte, J.L., Soulier, S., Persuy, M.A., Lepourry, L., Legrain, S., Printz, C., Stinnakre, G., 'LíHuillier, P. & Mercier, J.C. 1997. Application of Transgenesis to Modifying Milk Protein Composition. In : Milk

demand for speciality food proteins could create a market with a sufficient margin to justify the cost of recombinant dairy cows. However, there is at present little sign of such a market emerging. Usable results on this front may not emerge for many years because of the costs of transgenesis in cattle, the uncertainty of the economic benefits, and lack of knowledge of genes which can produce useful modifications. More precise physiological targets may benefit first.

In addition to extensive work on transfer of growth hormone in pigs.[39] Cysteine synthesis genes have been transferred into sheep with the objective of enhancing wool production[40] and a cold tolerance gene has been transferred from flounder into salmon.[41]

The second kind of target protein is one which is not part of normal functioning, but for which a farm species would be a convenient medium for production. One of the best known is human insulin, now produced routinely by transgenic bacteria. Parallel methods could be used to produce recombinant proteins in other species of micro-organisms, plants or animals. The choice of species is largely one of cost and efficiency.

Milking strains of cattle, sheep and goats have some advantages in this respect. They synthesise a range of proteins that make up about 40% of the solids in milk. Because of the large amount of research on the physiology of lactation, a great deal is known about the nature of milk proteins and their synthesis. Highly efficient production systems are already in place. The relative costs of production of a transgenic protein in bacterial culture, human cell culture and transgenic dairy cows have been calculated, and milking animals would be substantially the most cost effective.[42]

The potential for the synthesis of pharmaceutical products in milk is already being exploited. Of seven main products which are the focus of attention for this technology, almost all are currently derived from human blood. The total estimated market for these seven products was of the order of $US3 billion. However, the volumes required were such that (except for human albumin) a very small number of animals could supply world needs.[43]

The feasibility of developing transgenic vaccines for major diseases has been demonstrated experimentally for farm animals including poultry yet few if any are in general use. There have also been notable experimental successes with DNA vaccines which offer a very cost-effective means of distributing vaccines in many resource poor situations. A wide range of new vaccines are under development for animal diseases of importance to commercial animal production and it is likely that some at least will be developed to the point of practical application. In the longer term, it is likely that some of these vaccines can also be produced in transgenic plants using the same processes as developed for human vaccines.

The availability of breeds resistant to a disease would be expected to reduce the need for use of vaccines. Hence, the cost-effectiveness of the alternate options for disease management in

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Composition, Production and Biotechnology. 231-242. (Eds. Welch R.A.S *et al.*) CAB International, Wallingford.

[39]   Pursel, V.G. & Rexroad , C.E. 1993. Status of research with transgenic farm animals. J. *Animal Sci*. 71 (Suppl. 3) : 10-19.

[40]   Powell, B.C., Walker, S.K., Bawden, C.S., Sivaprasad, A.V. & Rogers, G.E. 1994. Transgenic sheep and wool growth : possibilities and current status. Reprod. *Fertil. Dev*. 6 : 615-623.

[41]   Hew, C.L., Fletcher, G.L. & Davies, P.L. 1995. Transgenic salmon : tailoring the genome for food production.*Journal of Fish Biology* 47 : (Supplement A) : 1-19.

[42]   Bremel, R.D. 1996. Potential Role of Transgenesis in Dairy Production and Related Areas. *Theriogenology* 45 : 51-56.

[43]   Wall, R.J. 1996. Modification of milk composition in transgenic animals. Proc. XX Beltsville Symposium : Biotechnology's role in Genetic Improvement of Farm Animals. 165-189. (Eds. Miller, R.H., *et al*.) USDA.

developing countries and particular diseases is an issue. Nevertheless, resistance to the pathogenic effects of disease agents without resistance to infection and dissemination of the agents could create additional problems concerning disease epidemiology and control, transmission to other species (including humans) and agent mutation. In addition for some diseases the ability to change the host *versus* the ability of the infectious agent to change can result in vaccine use forming part of the overall ongoing management strategy for these diseases.

The contribution made to improved disease diagnostics through the use of recombinant proteins and gene deletion mutant vaccines have already contributed significantly to disease surveillance and control programmes.

Because use of transgenesis is now such a major factor in many plant breeding programmes, it is important to see the much more modest expectations for application in animal breeding in the context of the different circumstances of plant and animal production. The first differences are in reproduction. In many plant species, tissue culture, gene insertion, clonal propagation are all well developed relatively low cost techniques. In animals, costs and technical difficulty are very much greater. Secondly, single gene traits, as for example for herbicide or insect resistance, are of clear and immediate economic importance. In animals, most traits of economic importance are multi gene traits, and therefore unlikely targets for gene transfer. Thirdly, the use of transgenics in plant breeding is driven by the huge financial returns made possible by the rapid and large-scale exploitation of the technology through annual seed sales. Animal populations (except for poultry) are replaced much more slowly, and the distribution channels of genetic material tend to be much more local, diffuse and farmer-based, and less easily used by major companies.

The role of intellectual property rights in the development of animal production technologies has so far been less than in plants. There is no equivalent of *sui generis* "plant breeder's rights"[44] over plant varieties, and the industry has so far felt no need for such protection, because animal "varieties" are very much fewer, not bred for individual traits, and less frequently replaced, and because animal reproduction rates are intrinsically much lower than in the case of plants. The high cost of maintaining quality herds, and of strict zoosanitary and quarantine requirements in themselves protect the investment of breeders. Few genetically modified animals have as yet entered the food production process, and patenting of production animals has not yet occurred on any large scale. Patenting of animals has so far been largely a phenomenon of medical and pharmaceutical research and production (for example, transgenic strains of mice), though this may soon change, if genetic marker technologies, such as marker-assisted selection, parentage identification, and gene introgression can equally be applied to livestock selection programmes.[45] Highly saturated genetic maps are now available for cattle, swine, and sheep to provide the genetic framework for developing marker assisted selection (MAS) programs. It is not yet clear what regulatory structures will emerge regarding the possible applications of transgenesis in farm animals, and for biosafety regulations for testing and releasing, and trade in genetically modified animals

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[44]   Such as provided for plants under the Conventions of UPOV (International Union for the Protection of New Varieties of Plants).

[45]   Davis GP, DeNise SK (1998) The impact of genetic markers on selection. J, Anim Sci. 76:2331-9.

3.4   Genetic Identification

The increasing knowledge of mammalian genetic structure, and the development of convenient ways of measuring that structure, have opened up a range of new possibilities in the areas of animal and product identification.

Parentage verification by livestock breed and registry associations has for many years been based on blood typing. This is now being replaced by typing based on microsatellite characterisation. Within a few years, the conversion to the DNA methodology will be complete.

The advantages of the new system are substantial. Better precision in identification should be possible, because the number of independent loci typed can be increased at will. The value of any particular locus depends on the number and relative frequencies of the alleles present in the population, as well as on the ease at which it can be amplified and read in the laboratory. The International Society of Animal Genetics (ISAG) is well advanced in the standardisation of panels of microsatellite loci in each of the main species.

A further advantage should be reduced cost, as automated methods replace conventional gel reading, and as DNA chip technology or mass spectrometry eventually makes gel based methods obsolete. Further economies are possible through the use of hair rather than blood in the provision of samples.

Producers of animal products, particularly meat, face increasing and legitimate demands from consumers for the greater guarantees of the integrity of the food production chain. This includes certification of production systems as well as guarantees related to the origin of the product. In the wake of the BSE crisis in Europe, this is of particular concern to beef producers.

Methods for using the new DNA technology to provide traceability in meat production have been developed.[46] The basis of the methods is the taking of a sample from the animal or carcass and its characterisation by a unique DNA profile. Any product derived from that animal can then be unequivocally linked to the animal by a matching DNA analysis. Current work is concentrated on refining these techniques and reducing costs so that they can be used to provide widespread consumer guarantees of traceability of product.

3.5   Molecular Conservation

The first step in considering the sustainable management or conservation of a particular population of animals is genetic characterisation. How unique is it in genetic terms? How different is it from other populations? How wide or narrow, and therefore how endangered, are its internal genetic resources? In the past, these questions could only be answered in very indirect ways. The development of efficient methods of reading the molecular structure of populations has added a totally new range of instruments which can be used for the development of rational and balanced genetic management strategies.

The most widely used of these techniques is the characterisation of a population at a range of microsatellite loci. These have the advantage that they are selectively neutral, and can therefore

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46   Meghen, C.N., Scott, C.S., Bradley, D.G., MacHugh, D.E., Loftus, R.T. & Cunningham, E.P. 1998. DNA based traceability for the meat industry. Proc. 8th World Conf. On Animal Production Vol II: 774-775.

reveal a great deal about the evolutionary history of the population, and of its relationships with other populations.[47]

In parallel, specifically maternal mitochondrial and paternal Y chromosome variation can be documented, and can shed additional light on patterns of gene flow between populations.[48]

The study of the gene complement of individual populations at the molecular level is becoming increasingly valuable for two reasons. The first is that as background information is built up, largely through the construction of the marker maps in the different species, the interpretation of new information becomes easier and more meaningful. The second is that with the rapid advance in technology, the scope, precision and cost of molecular characterisation is improving steadily.

In addition to general characterisation for conservation and management purposes, the identification of specific and useful genes, for example for disease resistance, is likely to identify resources which can be used to benefit populations other than those in which they have evolved.

In addition to the study of current genetic makeup of populations, it is also of value to establish DNA archives of well documented material representative, in particular, of populations which are endangered. This can be done simultaneously with sampling of breeds for molecular characterisation. The DNA archive can then act as benchmark material against which the success of management programmes aimed at the conservation of genetic variability can be monitored. Such a genetic archive for 104 European horse populations is now being established.

The FAO Global Strategy for the Management of Farm Animal Genetic Resources places strong emphasis on the use of molecular methods to assist the conservation of endangered breeds. A 1993 report[49] of a group of experts has been widely used by researchers utilising microsatellites to establish the genetic distances among breeds. This lead to the formulation of a global level programme of work known as the Measurement of Domestic Animal Diversity (MoDAD), involving a broad spectrum of researchers, co-ordinated at the global level by FAO and aimed at reliably establishing the pair wise genetic distances amongst breeds. The results of this increasing area of work, providing its conduct is effectively co-ordinated, will make important contributions to conservation decisions by countries and will also assist in breed development planning. An ISAG-FAO informal group of experts is identifying the common set of microsatellites which should be used in the research required for each farm animal species.[50] Although substantial research is underway for some species particularly in Europe and Sub-Saharan Africa, much work remains to be done.

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[47]   MacHugh, D.E., Shriver, M.D., Loftus, R.T., Cunningham, P. and Bradley D.B. 1997. Microsatellite DNA Variation and the Evolution, Domestication and Phylogeography of Taurine and Zebu Cattle (*Bos taurus* and *Bos indicus*). *Genetics* 146: 1071-1086.

[48]   Bradley, D.G., Loftus, R.T., Cunningham P. and MacHugh D.E. 1998. Genetics and Domestic Cattle Origins. *Evolutionary Anthropology* 6: 79-86.

[49]   Barker, J.S.F., Bradley D.G., Fries R., Hill W.G., Nei M., Wayne R.K. 1993. Measurement of Domestic Animal Diversity (MoDAD): Original Working Group Report. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans.

[50]   Bradley, D. G., Bumstead N., Cothran G., Crawfird A., Fries R., Nicholas F., Ollivier L., 1996. Microsatellite Markers for the Analysis of Genetic Distances in Domestic Animal Species. . In: Resources > Library > Reports of Meetings. URL http://www.fao.org/dad-is/

4. Recombinant hormones & Immuno-modulation

4.1 Bovine Somatotropin

Bovine Somatotropin (BST) was the first biotechnology product developed to modify livestock production. Somatotropin, or growth hormone, is a natural hormone secreted by the anterior pituitary in all mammals. Its structure differs considerably between species, and in the bovine consists of 191 amino acids. It has multiple physiological effects, and in particular it has major effects on the regulation of growth.

Forty years ago, it was first demonstrated that Somatotropin had a stimulating effect on milk secretion. It is now possible to transfer the DNA sequences responsible for Somatotropin synthesis into bacteria and to produce large quantities of Somatotropin relatively cheaply in bacterial cultures.

Many experiments have been conducted using genetically engineered BST, and over 1,500 scientific studies on BST had been published.[51] A summary of 21 experiments on almost 1,000 dairy cows showed that at all levels of treatment there was a substantial increase in milk production, ranging from 10% at the low levels (5mg BST per day) to 20% at the higher levels (20mg per day or more).[52] The percentage increase in production was relatively similar at all levels of production, and averaged about 15%. While some experiments have reported increases in fat percentage and marginal decreases in protein percentage, the general result is that BST administration has negligible effects on milk composition. The increase in efficiency from the spreading of the maintenance requirement over a larger output was about half the rate of increase in milk yield in the yield range 10-20kg/day, and about a quarter of the rate of increase in yield in the range 20-30kg/day.

Because BST is a new technology, there has been a degree of public concern about the safety of its use. The public health aspects of BST use in dairy cows have been subject to exhaustive evaluation in the USA[53] and it was authorised there in 1994 for commercial use. The European Union, on the other hand, has deferred consideration of commercial authorisation.

In all of the trials conducted so far, no specific negative effects on the cow have been found.[54] This element of technology can be compared to other technologies with a similar effect on production, for example, milking three times a day, or obtaining a 10% yield increase through breeding. In each case, higher production makes higher demands on the physiology on the animal, and if this is not supported with adequate feed supply, negative effects are observed on fertility in particular. Other health problems such as mastitis and ketosis have been found to be similar in BST treated and control animals. One unexpected effect of BST treatment is an increase in the frequency of twins.

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[51]   Bauman D.E. *et al.* 1994. International Dairy Federation Technical Report. *Bulletin of the International Dairy Federation* 299: 2-7.

[52]   Chilliard, Y. 1989. Long term effects of recombinant bovine somatotropin (BST) on dairy cow performances : a review. In : Use of Somatotropin in Livestock Production. (Eds. Sejrsen, K. *et al.*) EEC/ Elsevier pp 61-87.

[53]   Juskevich, J.C. & Guyer, C.G. 1990. Bovine Growth Hormone : Human Food Safety Evaluation. *Science* 249: 875-884.

[54]   Phipps, R.H. 1989. A review of the influence of somatotropin on health, reproduction and welfare in lactating dairy cows. In : Use of Somatotropin in Livestock Production. 88-119.(Eds. Sejrsen, K. *et al.*) EEC / Elsevier.

Eventual widespread adoption is predicted in developed countries, resulting in a reduction in production costs of the order of 7%. Most of this benefit is expected to flow through to consumers, with short term gains for those adopting the technology (generally the larger more efficient enterprise), and increased economic pressure on smaller producers. BST adoption is unlikely to be economic in milk production in most developing countries.

4.2   Immunomodulation

Higher vertebrates have evolved a complex defensive system. Molecules foreign to the organism ("antigens") are recognised and responded to. The response involves the recognition of antigens and production of neutralizing antibodies. Any technique which modifies the immune response comes under the heading of immunomodulation. However, the term has also been extended to include the induction of responses to antigens originating within the organism itself, e.g., hormones, or the use of passive antibodies against them to alter endocrine function. Within this second definition, a considerable amount of research has been devoted to the development of techniques for the alteration of fertility, growth, lactation and body composition in farm animals.

Strategies for immunomodulation may target the level of production of the hormone itself, the level of feedback controlling the hormone, or a hormone receptor. Antibodies may be presented by passive or active immunisation. Active immunisation involves the stimulation of the production of endogenous antibodies in the animal by exposure to small amounts of antigen. Passive immunisation involves the direct administration of antibodies which have been produced elsewhere. In general, active immunisation is preferred for long-term responses such as immuno-castration, while passive immunisation is more appropriate for eliciting short-term responses, for example, increased milk yield.[55]

Because the immune system is designed against foreign proteins, induced responses against endogenous proteins are generally weak. Much research has therefore been devoted to enhancing such responses. One approach has been to alter the antigenic molecule, generally by increasing its size. Another is to administer simultaneously immuno-stimulants ("adjuvants"). These have a double function: general provocation of the immune system and acting as a reservoir for antigen, thus spreading its effect.[56]

Ovarian function in sheep has been modified through immunisation against steroids and inhibin (a feedback hormone which controls production of follicle stimulating hormone). In general, substantial increases in ovulation rate (by 40 - 80%) were achieved. However, the increase in birth rates was about half of this, and the advantages were further reduced by higher losses *post partum*. The overall conclusion was that these techniques may have a role in improving fertility in a limited range of well-managed production systems.[57]

Immunisation against GnRH (Gonadotrophin - Releasing Hormone) in male and female cattle and in male pigs has been carried out. In males, the expected benefits are to combine the more docile behaviour of castrates with the growth rates of intact males. In pigs, the objective is also to avoid boar taint in meat. In females, the purpose is to suppress oestrus in heifers in feedlots and to reduce pregnancies in feeder females under range conditions. To varying degrees, all of these objectives

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[55]   Pell, J.M. & Aston, R. 1995. Principles of immunomodulation. Livestock Production *Science* 42 : 123-133.

[56]   Meloen, R.H. 1995. Basic aspects of immunomodulation through active immunization. Livestock Production *Science* 42 : 135-145.

[57]   Terqui, M., Wrathall, J.H.M., Driancourt, M.A. & Knight, P.G. 1995. Modulation of ovarian function by steroid and inhibin immunization. Livestock Production *Science* 42 : 181-192.

have been achieved in experiments. However, results were variable both in intensity and duration, and the general conclusion was that considerably more research is required.[58]

Increases in growth hormone (GH) are known to increase protein synthesis efficiency of feed use, as well as milk production in all mammals. The activity of GH and related hormones can be enhanced if GH is complexed with certain monoclonal antibodies. Work in mice showed dramatic increases in weight gains.[59] An alternative approach is to immunise against somatostatin (growth hormone release inhibiting factor). However, a review of experiments in pigs showed disappointing results.[60] Both of these approaches have been used to enhance GH action on milk yield. The limited number of experiments conducted in rodents, sheep, goats and cattle have not produced consistent results.[61]

A theme running through all of these reports is that each advance brings with it an increased awareness of the complexities of the hormone systems involved and of their interactions with the immune system. Furthermore, where favourable responses are obtained, they are often very variable, as well as partly offset by compensating losses. Nevertheless, several successful products (fecundin, vaxstrate) have been marketed to enhance animal productivity. Their impact, however, has been limited to very specialised production systems.

Immunomodulation could be said to fall between the enhancement of productivity by administration of exogenous hormones on the one hand, and permanent modification of the animal's hormone production pattern by transgenesis on the other. Since vaccination for health purposes is widely practised and widely accepted, the use of immunomodulation in production systems may face fewer problems in gaining acceptance than either of these other two technologies.

4.3   Feed Enhancement

There is already a substantial record of biotechnological contributions to livestock nutrition. These include single cell protein production, the genetic modification of nutrient value of forages, and the provision of a range of probiotic and antibiotic feed additives. In addition, the feed industry has introduced a range of enzymes for enhancing the nutritive value and quality of feeds. Furthermore, silage additives have been developed which include cellulase and hemi-cellulase enzymes as well as bacterial inoculates to provide the dual function of preservation and improvement in digestibility.[62]

Much of current research is focused on technologies for improving productivity in ruminants. Two well established technologies are the administration of ionophores and the use of microbial feed additives. The former disrupts bacterial membrane function and selectively suppress gram positive bacteria. This produces shifts in volatile fatty acid production and an improvement (average 7.5%) in feed conversion efficiency. The use of live microbial cultures, particularly of *aspergillus* and *saccharomyces* give benefits of similar order, though mainly through increased feed intake. Results, however, are variable, and the precise mechanisms involved are not fully understood.

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[58]   Bonneau, M. & Enright, W.J. 1995. Immunocastration in cattle and pigs. Livestock Production *Science* 42 : 193-200.

[59]   Holder, A.T. & Carter, C. 1995. Immunomodulation of the growth hormone - IGF - I axis. Livestock Production *Science* 42 : 229-237.

[60]   Elsaesser, F. & Drath, S. 1995. The potential of immuno-neutralization of somatostatin for improving pig performance. Livestock Production *Science* 42 : 255-263.

[61]   Flint, D.J. 1995. Immunomodulation of lactation. Livestock Production *Science* 42 : 201-206.

[62]   Robinson, J.J. & McEvoy, T.G. 1993. Biotechnology - the possibilities. *Anim. Prod*. 57 : 335-352.

Some 29 studies are on record in which genes from rumen micro-organisms had been cloned, mostly into *E. coli*, as well as 12 studies in which foreign genes (in all cases for antibiotic resistance) had been transferred into and expressed in rumen bacteria.[63] All of these studies are at fundamental level, and cannot be said to offer the prospect of usable technology in the short-term. The systems involved are enormously complex. Apart from the interactions of many rumen bacterial and fungal organisms and the host animal, there are other variables involving the feeds provided. The co-evolution of micro-organisms, animal and feed is likely to have produced balanced systems which would be difficult to disrupt with advantage. There may well be substantial differences amongst ruminant animal genetic resources and their rumen microbial genetic resources where these have evolved under widely differing production environments, but such complex genetic interactions have not been reported. Opportunities for genetically modifying the rumen microbial population may be greatest where new feed regimes are introduced. For example, high concentrate rations for ruminants are a recent introduction. These lower rumen pH, and there is therefore the prospect of improving fibre digestion by introducing cellulases into rumen bacteria which are tolerant of low pH.

Similar opportunities may exist where potentially useful plants contain toxic substances through the modification of rumen micro-organisms to reduce forage toxicity. In particular, the successful transfer of a detoxifying (dehalogenase) gene from the soil bacterium *Morexella* species to the rumen bacterium *Butyrivibrio fibrisolvens* has been reported. The genetically transformed organism was stable and functional in culture medium and also in the rumen of experimental sheep.[64]

4.4   Animal Genetic Resources and Biosafety

Questions of biosafety in animal biotechnology cover a range of issues, including, in particular, the regulation of the testing and utilization of transgenes in agricultural environments, immunology, and the trans-species transmission of disease. To date, there has been little application of transgenics in animal production environments. Most animal transgenics to date have been in medical applications (for example, human proteins incorporated in potential animal donors for xenotransplants, or genetically modified animals for medical research purposes), rather than in agriculture. Animals are also a promising route for the production of high value pharmaceutical proteins, where transgenic plants cannot fulfil the same function, and the advent of the cloning farm animals (with the sheep named "Dolly") arose from the desire to establish a methodology to reproduce asexually successful genetic constructs for such purposes. Because of the "contained" nature of most present uses of transgenic animals, with the lesser risk of unwanted escapes into the environment that this implies, and because the public seems intrinsically more tolerant of transgenics in a medical context than in agriculture, biosafety regulations for transgenic animals have received much less attention than have similar regulations for plants.

Partly because the vast majority of traits of interest in livestock improvement are polygenic, and because the option to apply a group of genes across species is less than for plants, transgenic technologies are currently not widely used in agricultural animal improvement programmes. However, recent advances in gene-mapping - including for cattle, swine, sheep and chickens - and in marker-assisted selection, make it likely that transgenic animals will be brought into production environments (including for modified milk) in the near future, which will require more systematic

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[63]   Wallace, R.J. 1997. Rumen microbiology and efficiency of digestion : Opportunities and Impact of biotechnology. In : Milk Composition, Production and Biotechnology. 465-489. (Eds.Welch, R.A.S. *et al.*). CAB International, Wallingford.

[64]   Gregg, K. 1995. Engineering gut flora of ruminant livestock to reduce forage toxicity : progress and problems. Tibtech 13 : 418-421.

consideration of the ethical and biosafety questions involved, and perhaps the harmonization of national testing and release regulations.[65]

5.   Conclusions

- Global output of the livestock sector, particularly in developing countries, is expected to double in the next 25 years. It is essential that most of this increase is achieved through improved efficiency rather than expanded numbers. A range of biotechnologies have important contributions to make in reaching these objectives.

  Domestic animals differ from agricultural crops in so many respects that the norms and conventions for both promoting and controlling technology will be substantially different in the two sectors. At the biological level, these differences are fundamental. While crops have a variety of reproductive mechanisms which can facilitate new technologies, animals have only one reproductive mechanism. This makes access to and intervention with the genetic material difficult and expensive. Much animal biotechnology is therefore centred on managing the reproductive process, and particularly on aspects of embryo production.

- Artificial insemination (AI) and embryo transfer (ET) are mature technologies for most farm animal species whose use has stabilised at approximately 50m. and 0.5m. globally. Most of this activity is in developed countries, and increased use in developing countries will depend on economic factors. It is important for both sustainable intensification and particularly as a low cost supplement for conservation but logistical and policy considerations for long term, reliable conservation genome banks are required particularly for developing countries.

- New and cheaper methods of embryo production *in vitro* may extend the use of ET, particularly if combined with new methods of sex separation of semen. However, commercially viable versions of these technologies are unlikely to be available for several years.

- Embryo cryopreservation is now well developed in cattle, and less so in sheep, buffalo, horses and pigs. For long-term conservation of genetic resources it is now a viable option in cattle, but not yet in the other species. Logistical and policy considerations for long term, reliable conservation genome banks are required particularly for developing countries.

- Recent developments in cloning of adult mammalian cells could have very large potential impact, particularly in dairy cattle. Most results would be beneficial through propagation of high performing and highly adapted genotypes. There is some potential danger from narrowing of genetic variation and decreased resilience in the face of disease challenge. Somatic cloning has important potential as a supplementary conservation procedure involving low cost, rapid sampling of animal genetic resources at risk in remote areas without the use of liquid nitrogen, but field protocols for sampling, temporary storage and transport of samples to central laboratories must first be developed and tested.

- The present rapid expansion of knowledge on mammalian genomes could have large potential impact on breeding programs. Direct identification of genes affecting important traits for economic performance or disease resistance, together with the use of neutral markers, could accelerate genetic improvement.

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[65]   For some discussion of the range of issues involved see Nilsson, Annika, 1997 (ed.). Transgenic animals and food production: proceedings of an international workshop in Stockholm. *Kungl. Skogs- och Lantbruksakademien Tidskrift* May 1997, 136, no. 20.

- The same technology can be used for the identification of individual animals and their products and therefore has great potential for improving traceability and integrity in the food chain.

- Molecular methods based on microsatellite assaying and genetic distance analysis are now established as important in the planning and management of genetic conservation programmes for endangered breeds. The co-ordination by FAO within the Global Strategy of the procedural aspects of genetic resource sampling, analytical methodologies and result reporting for the genetic distancing research activities which are getting underway for each animal species could help involve developing countries and rapidly realize cost-effective country and regional conservation decision making for the large number of animal genetic resources currently identified as at risk.

- Gene transfer has been used successfully to produce pharmaceutical products in animal tissues. This technology is unlikely to be of value for improving normal animal health or productivity because of cost, and because most traits of interest are multifactorial.

- The administration of recombinant hormones to enhance productivity in livestock has been led by the use of bovine somatotropin in dairy production. In countries where it is approved, it is now part of normal production technology, and extensive research has provided reassurance on animal and human health aspects. Economic factors concentrate its use in sections of high producing herds and limit its potential use in developing countries. Other physiological modifiers may follow, though questions of animal welfare and consumer acceptance are likely to limit their use.

- Immunomodulation aimed at modifying endogenous hormone function in the animal has been shown experimentally to improve fertility, growth, lactation and body composition in farm animals. However, results tend to be variable, and application is limited to a few specialised production systems.

- Many experiments have been conducted on methods of improving ruminant productivity through genetic modification of feeds, rumen micro-organisms and the animal. Results have been variable, and the complex interactions of breed, micro-organism and animal are often poorly understood. While the scientific potential of genetic manipulation has been clearly demonstrated, results usable on farm are not imminent.

- The inter-related questions of ethics and public acceptability have an important bearing on the extent to which scientific advances in biotechnology can be adopted in practice. These factors currently block the use of recombinant hormones and gene technology in animals in many countries. Concern surrounding these issues is likely to intensify, and those responsible for improving livestock productivity through research and application of new technologies need to engage in more active and balanced debate with other interest groups.

- The uptake of biotechnology in livestock production is determined by economic factors. It also generally requires sophisticated technological support. For these reasons, most developments in reproductive and genetic technology are likely to become established in the most economically advanced countries, with limited uptake in developing countries. These biotechnologies possess important potential for improving the effectiveness of both sustainable intensification and conservation of animal genetic resources.

- Policy issues related to conservation and biotechnology concern actions for protection and sustainable use of livestock populations and breeds; regulation of access to and ownership of genotypes; health, safety, welfare and ethical aspects of some new technologies; and provision

of open databases on animal genetic resources. These policy issues associated with the topic require substantial further development in both breadth and depth.

- In addition, future analysis of the topic could further detail the general development in this paper to explicitly describe the links between the manifold advances in biotechnology and the management of animal genetic diversity for each of the important farm animal species.