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THE STATE OF DEVELOPMENT OF BIOTECHNOLOGIES AS THEY RELATE TO THE MANAGEMENT OF ANIMAL GENETIC RESOURCES AND THEIR POTENTIAL APPLICATION IN DEVELOPING COUNTRIES

by

K. Boa-Amponsem[1] and G. Minozzi[2]

[1]Council for Scientific and Industrial Research
Animal Research Institute, P. O. Box AH 20, Achimota. Ghana.

[2] UMR Genetique et Diversité Animale, INRA
Jouy en Josas Cedex, France

The Third Session of the Intergovernmental Technical Working Group for Animal Genetic Resources recommended that FAO commission a study on recent developments in biotechnology, and current and potential use of biotechnologies, with a view to understanding constraints, especially for developing countries, in acquiring and using available biotechnologies relevant to the use, development and conservation of animal genetic resources. Developments in biotechnology in relation to the management of animal genetic resources were previously reviewed in Background Study Paper 10 (1999).

Dr Kwame Boa-Amponsem is the Director of the Animal Research Institute of the Council for Scientific and Industrial Research in Ghana (CSIR) and serves as the Technical Specialist with oversight responsibilities for Livestock, Fisheries and Agro-processing Research in the National Agricultural Research System (NARS) of Ghana. Giulietta Minozzi is a molecular biologist working at the Institut National de la Recherche Agronomique (INRA), France.

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1 Introduction

The world food economy is increasingly being driven by a shift of diets and food consumption patterns towards livestock products. A global surge in demand for animal protein is expected as a result of increased human populations, urbanization and increased incomes (Delgado *et al.*, 1999). Most of this will occur in the developing world. While per capita global meat consumption increased by 50 percent between 1964/66 and 1997/99, that for the developing world increased by 150 percent (FAO, 2003a). By 2030, per capita meat consumption could rise by a further 44 percent for developing nations compared with 13.5 percent for the developed world. There has also been a continued increase in the production of livestock products in the developing world over the past decade. Annual growth rates ranged from 3.7 to 9.4 percent for the period 1989 to 1999 (FAO, 2003a). Conversely, production in the transition countries actually fell, and grew only slightly in the developed world (ibid.). It has been projected that by 2030, developing countries' share of world meat and milk production would amount to 66 percent and 55 percent respectively. This trend notwithstanding, demand will grow faster than production in developing countries, producing a growing trade deficit (FAO, 2003a).

Increased production may be achieved through herd expansion and/or increased productivity. In most developing countries, small-scale farmers dominate animal production and depend chiefly on native breeds and free range production systems. A quarter of the world's land is used for grazing, and extensive pastures provide 30 percent of total beef production and 23 percent of mutton production (FAO, 1996). However, this is shifting. Land remains a finite resource for the whole world. In the developing world, grazing land is increasingly becoming unavailable as the need for infrastructural development for human dwellings increases. The more probable option for increasing animal production, therefore, is increased productivity per animal with consequent reduction in herd size. Such a reduction in herd size would minimize the deleterious effects of livestock farming on the environment. Increased productivity or efficiency refers to greater yields of animal products (carcass, milk or eggs) for the same unit of inputs.

Available data indicate that carcass weights have not increased uniformly among developing countries over the last decade (1989–1999). In Asia, where pressure on land is great, growth in herd size for cattle and buffalos was much lower than growth in output, whereas in sub-Saharan Africa the increase in cattle numbers was greater than the growth in carcass output. The scope for improvement is however phenomenal, considering that in 1997/99 the yield of beef per animal in developing countries was 163 kg compared to 284kg in developed countries (FAO, 2003a). The usual approach to improving performance parameters in native breeds in the past has been upgrading to high-performing exotic breeds through cross-breeding. Often, such programmes are not well designed, resulting in animals which are unsuitable for the environment and which require higher management levels than are affordable by small-scale keepers. The genetic dilution as a result of such programmes also denies well-adapted and 'less-demanding' livestock breeds to the local industry. A recent study of the Ghana Shorthorn indicated that the population of pure-bred animals formed only 47 percent of the total cattle population; down from 75 percent in recent history (Ahunu and Boa-Amponsem, 2001).

Thus, the maintenance of diversity in animal genetic resources and the simultaneous improvement of the productivity of native breeds kept under low input production systems, within the foreseeable future, present a challenge for animal improvement agencies in developing nations. Modern biotechnology has advanced remarkably over the last decade and has promise for improving the productivity of livestock by genetically improving important traits of the animals, their feed resources and their health care products. However, are these technologies ready for adoption within the context of low input production systems typical of many developing

economies, and what is their relative effectiveness compared with well-established conventional technologies such as pure-breeding schemes?

This paper reviews literature particularly that published after 1999 on the current state of development of genetic and reproductive technologies, and evaluates their potential impact on the utilization and conservation of locally adapted animal genetic resources (AnGR) for the sustainable intensification of production systems. Cunningham (1999) reviewed pertinent literature up to 1999. Country Reports prepared by countries in connection with the preparation of the First Report on the State of the World's Animal Genetic Resources provide information on the state of capacity of the various countries in relation to different biological technologies. In this paper, the term "biotechnology" refers to "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific uses" (Secretariat of the Convention on Biological Diversity, 1992

2 Reproductive technologies

2.1 Artificial insemination

Artificial insemination (AI) is the most widely used biological technology in developed and developing nations in livestock farming (Cardellino, Hoffman and Tempelman, 2003). Its notable benefits include the prevention of transmittable venereal diseases and the extension of the use of males of proven genetic merit in desired traits over a vast number of females, which would be unachievable through natural service. Frozen semen allows genetic progress to be disseminated worldwide. Today, artificial insemination is applied in cattle, sheep, goats, turkeys, pigs, chickens and rabbits (Foote, 2002). It has been most extensively applied in cattle production, particularly associated with dairy cattle where progeny testing is well established and demand for semen from highly valuable bulls is rapidly increasing. Continuing efforts are being made to refine methodology in order to reduce the number of sperms per insemination, and to improve the insemination technique itself. One of the problems limiting the use of AI in the beef cattle industry has been the difficulty with oestrus detection because cows are usually kept extensively.

Globally, more than 100 million AIs in cattle, 40 million in pigs, 3.3 million in sheep and 0.5 million in goats are performed annually (Thibier and Wagner, 2002). In chickens, its use is mainly limited to breeding companies, but unlike the larger species, fresh semen is used because cryopreserved poultry sperms are less fertile (Blanco *et al*., 2000; Blesbois and Labbie, 2003). AI is extensively used in turkey management where, because of the extreme sexual dimorphism, natural mating has become very difficult.

Facilities and extent of usage vary among countries. Country Reports indicate that several countries such as Cape Verde, Chad, Sudan, Cook Islands and Ghana started AI in the past but stopped due to financial constraints. Facilities have subsequently broken down. Some nations such as Bhutan, Laos, Congo Gambia and Guinea need the installation of new infrastructure in order to embark upon any meaningful use of AI.

Policy considerations for low input production systems which depended solely on imported semen from high performing exotic breeds would have to take cognisance of previous failed attempts to introduce AI. Reasons for the failure of AI in these countries include: lack of appropriately designed breeding programmes, inadequate economic incentives and technical difficulties. Typically, imported semen was intended to be used to upgrade native breeds to enhance performance in traits considered to be economically important in commercial agriculture. Several countries including Ghana, Cameroon, Sudan, Malawi, Botswana, Burkina Faso, Burundi, El Salvador, Uganda, Malaysia, Philippines, and Pakistan indicate gene flow into the

livestock sector through AI from exotic semen. Breeding programmes failed because of unclear objectives, use of semen from unadapted breeds, station-bound uncoordinated cross-breeding and absence of breeding structure for the livestock industry. Use of AI within the context of an ongoing, appropriately-designed, national or regional breeding programme would minimize maintenance costs for farmers and give the programme a solid footing.

A major cause of failure of AI has been the lack of economic incentives for its adoption. The peri-urban small scale dairy project in West Africa during the late 1980s and the current FAO-promoted "Village Milk System" have not achieved the expected impact, mainly because of limited markets, particularly for liquid milk (Lambert *et al.*, 2004). This is rather an irony considering that these nations import annually huge amounts of milk (FAO, 2003a). Large multinational processing companies prefer imports, and rarely enter into contractual supply arrangements with local producers (Lambert *et al.*, 2004**).** Finally, policy should consider technical support for implementing AI technology. In a recent electronic forum organized by the FAO (FAO, 2001), major concerns were expressed by participants about technical difficulties with AI (heat detection, high cost, sustainable supply of components such as liquid nitrogen, and poor communication). The technology could be introduced into more peri-urban areas initially, and diffused gradually into more remote areas. Formation of community farmer groups and the use of solar batteries have facilitated the extension of needed animal husbandry services to remote rural areas in some developing nations.

Policy considerations in the highly-developed dairy cattle sector should take into account the erosion of genetic diversity between and within breeds, considering that 50 percent of almost 5 000 Holstein bulls born in 1990 in 18 countries were bred by only five sires (Wickham and Banos, 1998). Clear strategies for enforcement of policy decisions should be also presented in order to reduce the erosion of genetic diversity.

2.2 Semen Sexing

Sex determination of semen, which enables the preferential production of one sex, is of major interest for all animal species used for food and agriculture. Newly hatched male chicks from layer breeders are usually killed immediately after hatching because of their slow growth rate and inferior meat characteristics compared with broilers. The number of off-sex chicks killed annually in the EU and US are estimated to be 282 and 226 millions respectively (Ellendorff and Klein, 2003). Producers of breeding stock, feedlot operators, herd replacement by farmers and several segments of the livestock industry stand to benefit immensely from this technology. The reductions in herd or flock size that are possible with this technology will minimize the detrimental impact of animal production on the environment.

The method used to sex semen is based on the difference in the DNA content of the X (Z in avian species) and Y (W in avian species) sperms. The X chromosome contains 2–5 percent more DNA, depending upon the species. DNA contained in the gametes is treated with fluorescent DNA binding dye. Flow cytometry and cell separation follows, based upon the different fluorescence emission. Semen is analysed in series, singularly, and divided at a speed of about 3 000 live spermatozoa per second of each sex; in some cases, rates may be increased under optimal conditions (Seidel *et al.*, 2002). In swine some positive experimental results have been published. Sorted semen was deposited intratubally in two gilts, which subsequently farrowed 13 of 15 (85 percent) piglets of the predicted gender (Rath *et al.*, 1997). Calves from sexed spermatozoa had no more abnormalities than those of controls, and growth was similar for both groups (Tubman *et al.*, 2003). Sex-selected bull semen is commercially obtainable in some developed countries (Holt, 2003).

2.3 Embryo transfer (ET)

ET in domestic farm animals enhanced by multiple ovulations and the freezing of embryos enables the extended use of superior female genetic material, and whole genomes (diploid) across continents at low transportation costs. Initially, surgical procedures were used to obtain embryos from valuable donors. Today non-surgical procedures are used routinely in commercial application. ET is the major vehicle for the regeneration of rare breeds for which conserved embryos are available. It could also be used to increase their population sizes. It still remains the principal tool for the application of more advanced reproductive biotechnologies such as ovum pick up, sexing of embryos, cloning and genetically modified animals.

In 1998, globally 440 000 ETs were recorded in cattle, 17 000 in sheep, 1 200 in goats and 2 500 in horses, while 80 percent of the bulls used in AI in the developed world are derived from ET (FAO, 2001). In the developing world its use in well designed cross-breeding programmes would enable high-input production systems to use exotic stocks without diluting the genetic diversity of local populations. Indications are, however, that the use of ET is on the decline worldwide as a result of its high cost (Schmidt, F., 2004; personal communication). Thus, the future use of ET and its widespread adoption by developing countries will depend upon further refinements in methodology which reduce costs. Embryo Transfer (ET) is rarely applied in Africa but research efforts to use it in cattle are ongoing in some countries such as Burkina Faso (see also Alhassan, 2003) and Madagascar. ET is used mainly at the experimental level or on a few commercial farms in certain Eastern European countries including Hungary, Slovenia, Poland, Croatia and Czech Republic. In Latin America (e.g. Brazil and Mexico), it is used commercially but to a rather limited extent because of its high cost. Bovine ET in Brazil amounts to 82 000 annually.

2.4 Embryo Production through *In Vitro* Fertilization (IVF)

The production of a large number of offspring from females of high genetic merit, involving procedures of oocyte collection through Ovum Pick Up (OPU) directly from the ovary can potentially accelerate genetic progress by increasing the selection intensity and reducing the generation interval. This technology is more advanced in cattle production. Originally, oocytes were obtained from slaughterhouse material, although there were many disadvantages. Today an increasing portion of oocytes used for embryo production by *in vitro* techniques are from live donors. The technique, referred to as ovum pick up (OPU), is based on the trans-vaginal recovery of the oocytes by aspiration of the ovarian follicles with the aid of an ultrasound probe. The recovered oocytes are matured and fertilized with semen of the selected sire and developed to the compacted morula or blastocyst stage in the laboratory. The process is referred to as *in vitro* maturation and *in vitro* fertilization (Besenfelder, Muller and Brem, 1998). Subsequently the embryos can be transferred fresh into a synchronized recipient or can be frozen.

The application of sexed semen technology coupled with IVF appears to provide the most logical and first commercial application for sexed semen. The inherent cost of separated sperm fits well into commercial IVF schemes, where small quantities of sperm are needed to achieve fertilization. On average, it is recommended to use 2 million sexed sperm to inseminate a single heifer. In contrast, less than 100 000 sperm can effectively fertilize 100 oocytes *in vitro*, thus reducing the problem of low sperm numbers following sexing. The potential to separate frozen–thawed sperm would provide additional opportunities for the IVF production of embryos. However, further improvements will need to be implemented for this technology to gain widespread use in the dairy industry.

OPU is very flexible and can be performed repeatedly on donors of almost any physiological status including milking, pregnant and pre-pubescent females. Conversion rate from oocytes to transferable embryos in cattle remains rather low (less than 30 percent). Thus the cost of the

technology is justified only where an individual animal is valuable as in the cases of cattle, horses and buffaloes. Refinement of current procedures to reduce costs will make the technology accessible to users in developing nations for rapid dissemination of genetic progress being made in breeding centres. One suggested idea is to collect ova from cows culled in industrialized countries and perform IVF with semen from local bulls in developing countries to maintain a continuous population of F1 commercial animals. Benefits could be an immediate increase in genetic potential, and maintainence of a continuous supply of F1 animals, perhaps at a reasonable cost.

2.5 Embryo sexing

Sexing of embryos has great potential for the livestock industry. If the sex ratio of the progeny can be pre-determined before transfer and implantation, the number of cows in the breeding herd can be reduced. The sexing technique is based on the chromosomal difference that exists between embryos of different sex, determined by the presence or absence of the Y chromosome. In order to highlight this difference, a small group of the cells of the embryo is removed. The DNA contained in the cells is extracted and amplified with Y chromosome specific DNA probes through PCR (Polymerase Chain Reaction).

Among the methods used for embryo sexing such as karyotyping, antibodies specific for male antigen, X-linked activity enzymes and the use of specific probes for the Y chromosome, amplification of Y chromosome-specific DNA by means of the PCR technique mentioned earlier, seems to be the most reliable and practical method (Shan-Nan Lee, 2000). This method is 90 percent accurate.

2.6 Cloning (Non-Sexual reproduction)

Clonal propagation is the norm for crops such as potato and cassava, and somatic embryogenesis in the plant kingdom was demonstrated several decades ago. In the animal arena, the advent of successful cloning of farm animals has become technically feasible in the 1990s with the birth of "Dolly". Yet there is a great interest in the production of two or more genetically identical individuals or clones. Cloning provides uniformity of end-product to meet demands of a traditionally stable market where the potential return is high. In basic scientific and medical research, cloned animals are of very high value because they show very limited genetic variation. Its potential in multiplying transgenic founder animals and rare breeds is enormous. Clones of highly adapted and high- performing animals generated from well designed breeding programmes in low input systems, would be invaluable for the high stress but stable environments typical of some developing countries. Subsequent improvement in the performance of clones and response to changing market and disease situations, however, remain issues, but cloned sires used on a random sample of females in the population would still retain enough variability in the progeny to allow for further improvements. The viability of somatically cloned animals, however, has been questioned following the death of Dolly. Post mortem findings indicated that Dolly had sheep pulmonary adenomatosis (SPA) and arthritis (Griffin, 2004). Dolly's telomeres were slightly shorter than would be expected in a sheep of her age conceived naturally. Other studies have shown cloned animals to have normal to slightly below normal life expectancies (Cibelli *et al.,* 2002; Pace *et al.,* 2002; Tian *et al.,* 2005; Wells *et al.,* 2004).

Methods of cloning include:

- Embryo splitting
- Somatic cell nuclear transfer
- Stem cell nuclear transfer

2.6.1. Embryo splitting

Embryo splitting generates completely identical animals. It is performed by surgical bi-section of early tubal stage embryos, morulae and blatocyst stages (Besenfelder *et al*., 1998). After blastulation the inner cell mass is divided into two equal parts and the resulting zona pellucida-free demi-embryos are transferred to recipients. The split embryos can be either cultured *in vitro* or transferred into foster animals immediately after micromanipulation. Published successes with this method remain few, with vast variation between species. For instance, pig embryos seem to be more sensitive to the splitting procedure than bovine embryos (Besenfelder *et al*., 1998).

2.6.2. Somatic cell nuclear transfer

Somatic cell nuclear transfer (SCNT) technology allows the generation of clones of animals about which substantial knowledge of their performance exists, which is an advantage in matching breeds to specific market or environmental niches. Cloned sires with superior genetics can be used for natural mating in remote areas of developing countries where AI is impracticable. The technique fuses a nucleus of a somatic differentiated cell, after reversing the DNA quiescence, with an enucleated and unfertilised egg. The embryo is then implanted into a foster or surrogate mother. The first species to be cloned by this procedure was the sheep Dolly, in 1996 at the Roslin Institute in Edinburgh (Wilmut *et al.,* 1997). The same technique has been applied to produce, with success, offspring from mice, cattle, goats, pigs, rabbits, horses, rats, mules and cats. Restoring nuclear totipotency in already differentiated somatic cells and the following NT procedures in order to produce live offspring is still a very inefficient method. Dolly was the only live offspring produced after 277 attempts (Wells, Oback and Laible, 2003; Edwards *et al*., 2003). Although the technique does not imply transgenesis, it can be used with genetic modification to produce genetically modified animals (GMOs). The storage of somatic cells is now being used as a low-cost way of establishing a gene bank, in the hope that technologicial advances will make cloning feasible.

2.6.3. Stem cell nuclear transfer

Embryonic stem (ES) cells are derived from totipotent cells of early embryos and have the potential for unlimited and undifferentiated proliferation when in culture (Evans and Kaufman, 1981). The potential uses of the ES cells are: to clone a larger number of animals from a single embryo and to introduce specific and targeted genetic modifications. The use of this technology in farm animals has not been popular due to the difficulty in creating ES cell lines in cattle (Cibelli *et al.,* 1998). However, a resurgence of interest has been predicted because ES cells are more amenable to precise genetic modifications, which result in higher cloning efficiencies in mice and ensures a more rapid dissemination of genetic improvements compared with somatic cell cloning (Wells, Oback and Laible*,* 2003; (Jaenisch *et al.,* 2002).

If cloning becomes feasible and ethically acceptable, it could be used to start bull exchange programmes as a forerunner to a fully functional AI programme in developing nations. In such a programme, bulls from cloned sires would be supplied to farmers in exchange for their animals, as payment. Also, using high selection intensities, a few top sires and dams could be cloned to be used directly for production to match specific environmental or market niches with little effect on genetic diversity, since the rest of the population would be improved using conventional breeding methods. It is, however, estimated that it will be some years before the cloning practice is used on a commercial scale even in developed nations, while research continues to find the most cost effective methodology that also avoids negative effects on animal genetic resources (Biolatti and Appino, 2000).

2.7 Male germ cell transplantation

This is an method of tissue transplantation in which germ cells are retrieved from a donor testis and transferred into the testis of a recipient animal. In contrast to rodents, immunosuppression does not appear to be required in pigs and goats, in which unrelated donors and recipients have been successfully used (Honaramooz *et al*., 2002). Transplantation of bovine germ cells between breeds of cattle can be successful (Hill and Dobrinski, 2006). Testis cells from *Bos taurus* bull calves were transplanted into *Bos indicus* x *Bos taurus* cross-bred bull calves to produce tropically adapted bulls that will produce *Bos taurus* sperm and, when mated to *Bos Indicus* cows, F1 progeny. Although the primary reason for most testes transfer research in livestock is to assist in the dissemination of superior genetics, testes transfer may also offer the opportunity to reduce the generation interval on the male side (Henshall et *al*., 2006).

3 Cryopreservation

Cryopreservation refers to the storage of gametes (haploid genomes), embryos (diploid genomes) and other biological materials for future use. It is crucial for the conservation of threatened species and breeds for maintenance of biological diversity and modern breeding management of some domestic farm animal breeds. Cryopreservation enables the shipment of genotypes of proven genetic merit across the world at less cost, to be utilized through AI and ET technologies. There are many differences today in the feasibility and practicality of the cryopreservation of different biological materials that carry different genetic information. In addition, complete documentation of the stored material is essential for all potential purposes.

3.1 Gametes (Haploid genome)

3.1.1. Semen

Semen can easily be collected in certain species and preserved through slow freezing protocols. Long-term storage requires liquid nitrogen. The collection and storage of semen is used routinely for cattle, pig, sheep, goat and horse (see Table 4.1) production. Cryopreserved ram semen has high conception rates when used with laparoscopic intra-uterine insemination rather than intra-cervical AI (Holt, 2003; Hill *et al*., 1988). Chicken semen has only been frozen successfully experimentally, using rapid cooling with dimethyl acetamide to achieve high fertility rates (Tselutin *et al.,* 1999). Turkey spermatozoa are much more subject to irreversible damage in response to cooling/freezing than chicken semen (Blanco *et al*., 2000). Frozen semen can be used to recover a lost breed through at least six generations of back-crosses, starting from a group of females of another breed (Ollivier and Renard, 1995). When storing semen as a biological material, mitochondrial genes will not be preserved, and only a single component of the chromosomes is preserved (Woelders, Zuidberg and Hiemstra, 2003).

3.1.2. Oocytes

Oocytes are extremely sensitive to chilling and are difficult to cryopreserve. At present, research on cryopreservation of oocytes is not sufficiently advanced to enable the use of oocytes as storage material on routine basis (Woelders, Zuidberg and Hiemstra, 2003). Many benefits could derive from successful cryopreservation of oocytes, two of which are that mitochondrial genes are not lost, and that when stored with semen, no backcrossing is required.

Table 3.1 Effectiveness of insemination with deep-frozen semen by species

Species	Birth Rate following AI	Specimen containers per Production (n)	Productions per week (n)	Comments
Horse	40 – 50%	20 – 30	2 – 4	Relatively high proportion of premature embryo mortality
Cattle	50 – 60%	300 – 1000	2	
Sheep	55 – 60%	10 – 15	3	After insemination of female animals (synchronised oestrus), the pregnancy rate with frozen semen is only 10 percent lower than that achieved with fresh semen
Goat	60%	10 – 12	5	High fertility – kidding rate: 76 percent, with semen obtained in the second half of the breeding season.
Pig	60%	8 – 10	1 – 2	Greatly improved thinners have allowed longer shelf-life and a greater increase in pig insemination in recent years.

Source: Country Report for Germany.

3.2 Genotypes (diploid genomes)

3.2.1. Embryo

Embryo collection and subsequent storage are possible for many species. Embryos are the best choice if the purpose of the collection is to restore a desired genotype, although it is very expensive. Two methods are currently used for embryo cryopreservation: slow freezing and vitrification. Vitrification technology avoids cell death caused by intracellular ice formation. Vitrification is particularly suitable for sensitive embryos such as those of pigs (Dobrinsky *et al*., 2000). Storing embryos enables the conservation of mitochondrial genes. Cryopreservation of cattle, sheep and goat embryos is successful (Table 4.1) and, hence, systematic embryo conservation programmes are realistic. With horses, due to the lack of super-ovulation effects the yield is so low that the costs of practical conservation programmes would be extremely high. Avian and fish embryos have not yet been successfully cryopreserved because their eggs contain large amounts of vitellus (Blebois and Labbe, 2003).

3.2.2. Somatic cells

Somatic cells can be easily cryopreserved, and the procedure is cheap. It is widely used for research purposes because both defrosting and DNA extraction follow simple protocols. The subsequent usage of this frozen material is, however, very expensive. Live offspring have been obtained after cloning procedures in sheep, cattle, mice, pigs, goats and rabbits (Lai *et al*., 2002). This is a particularly attractive option for conservation activity involving animal genetic resources at risk in remote rural areas where sampling and storage of adequate samples of semen and embryos is not practical. Its importance was underscored by a team of experts supported by FAO and the Italian government who evaluated the opportunities and needs for the use of somatic cloning in the conservation of AnGR (FAO, 1998).

Table 3.2 Status of embryo conservation in domesticated animals

Species	Embryos/Superov./ Production in Cattle including OPU/IVI	Production Conditions	Optimal Stage For Conservation	Number of Offspring per Frozen Embryo
Horse	1 (low SO reaction)	Transcervical, can be repeated regularly	Morulae-young blastocysts	Approx. 40 – 45 %
Cattle	4 – 6	Transcervical, can be repeated regularly	Morulae– blastocysts D6.5-D8	Approx. 60 %
Sheep	4 – 5.5	Surgical; laparoscopy season-dependent	Morulae- blastocysts	40 – 65 %
Goat	4 – 8	Surgical or transcervical; season-dependent	Morulae-exp. blastocysts	35 – 55 %
Pig	15 – 20	Surgical	Morulae- blastocysts	Approx. 10 – 20 %

Source: Country Report for Germany
AI: Artificial insemination
OPU/IVI: Ova pick up/in vitro insemination
SO: Superovulation

3.3 Cryobanks

Most cryobanks store materials from both threatened and non-threatened populations. The main goal in managing a conservation programme is to prevent extinction of threatened populations. In this case, cryopreservation allows *ex situ* and *in situ* conservation to complement each other. In species such as cattle, where allowing females to breed freely may be essential, it may be easier and cheaper to use frozen semen to reproduce the populations (Verrier *et al*., 2003). The crisis caused by the foot-and-mouth disease epidemic of 2001 in the United Kingdom has demonstrated that breeds comprising large numbers of individuals and commercially farmed can be at considerable risk of extinction. Natural disasters and wars, similarly, cause the extinction of breeds. Hence, many cryobanks also store materials from non-threatened populations.

Non-threatened populations are of two types: animals representing original and/or extreme genotypes on one hand and those representing the current state of the population undergoing genetic selection on the other (Verrier *et al*., 2003). Extreme animals for a particular trait or for an identified locus may be lost due to chance or may be selected against where a global trait (e.g. an index) is the breeding goal. Such individuals together with those of an original pedigree line are invaluable in restoring lost genetic variability. A comparison between current performance levels and those of control populations of poultry species indicated the extent to which artificial selection had altered the original breeds (Havenstein *et al*., 2004). It is now regular practice for animals from ongoing commercial populations to be included in cryobanks. Cryobanking, thus, gives the opportunity to occasionally "freeze", a sample of a population undergoing artificial selection, to be used if changes in demand necessitate changes in breeding goals, or simply to evaluate genetic progress as it is not always (economically) possible to keep control populations. It is highly recommended that samples of biological materials should be kept in more than one cryobank, especially in developing nations where stable sources of electric power cannot be guaranteed.

Ownership and use of biological material from a cryobank are covered by legal instruments which must be adhered to by both donors and recipients. Mutual agreements on the terms and conditions of the transfer including a fair and equitable sharing of the benefits arising out of their utilization have been provided for by guidelines of the various articles of the Convention of Biological Diversity (www.biodiv.org – UNEP/CBD/COP/6/6).

Cryopreservation facilities vary widely among nations according to Country Reports. In several European nations (Planchenault, 2003), national gene banks are encountered in addition to those held at AI centres, universities, commercial breeding companies and private interest groups. Cryopreservation is hardly practised in the Near East and Africa with Benin being the exception. Only a few nations in Latin America (e.g. Brazil) and Asia (e.g. Japan, China) have *ex situ* conservation in the form of cryobanks. The establishment of regional cryobanks is an important policy consideration. Initially, these could be stocked with semen from those species with limited reproductive ability and for which *ex situ* conservation must provide a back up for *in situ* conservation schemes. However, the legal framework to regulate the operation of such gene banks across nations needs to be carefully drafted to accommodate the concerns of all stakeholders and maintain transparency. This technology could then be within the reach of such nations in the foreseeable future (less than 5 years) with international financial and technical support.

4 DNA-Related Technologies.

All living organisms are made of cells whose functions are controlled by genetic material called DNA. This molecule is made up of a long chain of nitrogen-containing bases (there are 4 different bases - A, C, G and T). A specific sequence of a number of these bases defines a particular gene. The length of a DNA fragment is measured in base pairs (bp). The haploid mammalian genome contains 3 billion base pairs and encodes about 30.000 genes. In a diploid individual where chromosomes are organized in pairs, there are two alleles of every gene- one from each parent. A functional gene that codes for proteins consists of sequences that are transcribed into mRNA (messenger RNA) (subsequently translated into proteins) and those that make up the regulatory elements. It is now estimated that as little as ten percent of the mammalian genome is functional, in the sense that it specifies transcription or its regulation and only 2–3 percent of the genome consists of exons that, thus, specify transcription (Moran, 1998).

One way to study the expression of a gene is gene silencing. Double-stranded RNA-mediated interference (dsRNAi) is a simple and rapid method of silencing gene expression in a range of organisms. The silencing of a gene is a consequence of the degradation of RNA into short RNAs that activate ribonucleases to target homologous mRNA. Specific gene silencing has been associated with regulatory processes such as antiviral defense mechanisms, gene regulation, and chromosomal modification. dsRNAi is being widely adopted in research and therapeutics. It is rapidly replacing conventional gene knock-out technology.

These insights into the structure and functioning of the genetic make up of living things have fuelled a phenomenal accumulation of knowledge, which has implications for animal agriculture particularly for developing nations.

4.1 Molecular markers

A DNA marker is an identifiable DNA fragment or sequences which can detect a DNA polymorphism. These are found at specific locations in the genome and can be considered as stable landmarks in the genome. They are transmitted from generation to generation by the standard laws of inheritance. When located close to a functional gene, they can then be used as a

marker for the functional gene. Different kinds of molecular markers exist, such as RFLPs (restriction fragment length polymorphisms), RAPDs (random amplified polymorphic DNA) AFLPs (amplified fragment length polymorphisms), microsatellites and recently SNPs (single nucleotide polymorphisms). Korzun (2003), considering the case of cereals, provided a comparison of these marker systems (Table 4.1)

Table 4.1 A comparison of the major molecular markers (Korzun, 2003)

Feature	RFLPs	RAPDs	AFLPs	Microsats	SNPs
Amount of DNA required in mcg	10	0.02	0.5-1.0	0.05	0.05
Quality of DNA required	high	high	moderate	moderate	high
PCR-based	no	yes	yes	yes	yes
No. of polymorphic loci per analysis	1.0-3.0	1.5-50	20-100	1.0-3.0	1.0
Ease of use	not easy	easy	easy	easy	easy
Amenable to automation	low	moderate	moderate	high	high
Reproducibility	high	unreliable	high	high	high
Development cost	low	low	moderate	high	high
Cost per analysis	high	low	moderate	low	low

There are many potential current and future applications of molecular markers, ranging from the measurement of genetic diversity in genetic distance studies, MAS (Marker assisted Selection), MAI (Marker Assisted Introgression), to their use in genotype verification such as in traceability protocols utilizing microsatellites. Another application of molecular markers is found in the detection of QTL (Quantitative Trait Loci) in order to localize genes that contribute to genetic variation. The above mentioned examples along with other applications of molecular markers are discussed in further detail below.

4.2 Measurement of genetic diversity

Because of changing market demands, diversity of AnGR in the developing world is rapidly being lost through breed replacements and lack of incentives for breed development and, hence, breeding programmes. It is, for economic reasons, not possible to conserve all breeds in danger of extinction. Hence, genetic distances or relationships within and between breeds need to be established to ensure that those conserved are genetically the most distantly related. Some of the most frequently used markers for genetic distance studies are microsatellites, and in recent times, SNPs.

Many diversity studies for livestock species have been carried out in both developed and developing countries, and have frequently utilized FAO-recommended procedures and microsatellites for the Measurement of Domestic Animal Diversity (FAO, 1993; FAO, 1995), except in studies with chickens where several laboratories preferred to use their own lists, claiming that they yielded better information (FAO 2004a). In future, SNPs will compete with microsatellites as the marker of choice.

Mitochondria are particularly useful in studying patterns of gene flow between populations (Rothschild, 2003) and, hence, are an important tool for evolutionary and genealogical studies in livestock. Mitochondrial sequence analysis was used in a genetic distance study involving 13 cattle breeds that included West African *Bos taurus* and Zebu breeds, European and Asian breeds (Loftus *et al*., 1992). The results indicated that there were two mitochondrial lineages: one representing European and African mitochondria including Zebu breeds, and the other representing Asian *Bos indicus* mitochondria. No further substructuring could be detected between the European and African groups. One of the problems of this technique is the presence of mitochondrial pseudogenes in the nuclear genome in some species (Bensasson *et al*., 2001)

Microsatellites are simple DNA sequences, usually 2–5 bases long, repeated a variable number of times in tandem. They are easy to detect with PCR (polymerase chain reaction), a molecular biological procedure that allows the production of multiple copies (amplification) of specific DNA sequences. Microsatellites are the most polymorphic markers. The repeat unit for most mammals is AC/GT (Crawford and Littlejohn, 1998).

Single nucleotide polymorphisms (SNPs), i.e. single base changes in DNA sequence, have in recent years become an increasingly important class of molecular marker. The potential number of SNP markers is very high, meaning that it should be possible to find them throughout the genome, and micro-array procedures have been developed for automatically scoring tens or hundreds of thousands of loci simultaneously at a low cost per sample (FAO, 2003b).

Mitochondial genome. Eukaryotic cells contain a class of cytoplasmic DNA molecules which are found only within the mitochondria where they replicate and are transcribed. This mitochondrial DNA (mtDNA) constitutes the "mitochondrial genome" and carries genetic information essential to mitochondrial function. In view of its extranuclear location, it is not surprising that inheritance of the mitochondrial genome is not governed by the same rules that apply to chromosomal genes. There is evidence that the mitochondrial genome of an individual is derived solely from the maternal parent (in contrast to chromosomal genes, which are inherited biparentally).

4.3 Marker Assisted Selection (MAS)

An important use of the molecular marker system is for the construction of DNA marker (genome) maps with many markers of known location interspersed at relatively short intervals throughout the genome. This has been done for most economically important farm animal species. Markers to aid selection (MAS) have been sought for a long time, and in the past biochemical, blood groups and clinical markers have been used (Neimann-Sorenson and Robertson, 1961). The usefulness of markers is that because they are linked to traits of interest, gene frequency of these traits can be acted upon by using these markers. Two categories of traits are involved: monogenic traits where a single locus determines the phenotype and polygenic traits resulting from the combined effects of several quantitative trait loci (QTL) and the environment.

Major advantages of MAS include the following:

- A more accurate selection can be performed due to the additional information provided by the markers.
- It allows for higher selection intensities because of an increased number of candidates.
- It allows for selection for traits expressed in one sex.
- It allows for selection for traits that normally necessite sacrificing some of the animals (carcass traits).
- It allows for selection for lowly-heritable traits where phenotypic measurements are less valuable.
- Earlier selection and consequently shorter generation intervals are made possible as markers can be typed early in life and long before the trait can be measured. This will be an advantage in species with long generation intervals.

The effectiveness of markers depends upon how tight the linkage is with the trait genes. Where the linkage is tight, they will be inherited together. If it is located within the gene, it is the most effective (gene assisted selection). Theoretical predictions made about the efficiency of MAS often rest on the assumption that the associations between markers and quantitative traits are the result of linkage disequilibria and are likely to be inherited together (Ollivier, 1998). The most difficult situation for applying MAS is where there is linkage equilibrium. Predictions about response to MAS are generally for only one generation since recombination is expected to progressively decrease the efficiency of the selection. The current cost involved in DNA collection, genotyping and analysis, apart from the cost of QTL detection, would need to fall considerably for it to be considered a real alternative to conventional breeding schemes. Dekkers (2003) recently reviewed commercial applications of MAS in livestock and concluded that the high initial expectations for the use of MAS have given way to an attitude of cautious optimism.

The idea of shortening the generation intervals in MAS schemes was taken to the extreme in the so-called velo (George and Massey, 1991) and whizzo (Haley and Visscher, 1998) schemes by harvesting oocytes from calves while still in utero. The number of selection cycles in a given time period is minimized, which may increase rates of genetic gain dramatically. The genomic selection was proposed (Meuwissen, 2001) to obtain a high accuracy of selection in in-utero calves or early embryos.

4.4 Marker Assisted Introgression (MAI)

Another use of markers is for introgressing individual genes from one breed into another through repeated backcrossing to a recipient breed. Markers are used to select individuals expressing the gene for backcrossing and thereby reduce the number of generations required. This has been exploited in introgressing the halothane–resistance allele into the Piétran breed of Belgium (see review by Ollivier, 1998). In situations where the donor breed is inferior, there is a need to recover as quickly as possible the genomic background of the recipient breed. Both DNA fingerprints and microsatellite markers could be used in a process referred to as *genomic selection (GS)* to accelerate the recovery of the genomic background of the recipient breed. By selecting individuals with maximum genomic similarity to the recipient line at each cycle of backcrossing, the recipient genome can be recovered through fewer cycles of backcrosses. Under an optimal density of two or three markers per morgan, and selecting a proportion of 10 percent on the markers per generation, 98 percent of the recipient genome can be recovered with two fewer generations using GS (Ollivier, 1998).

The relative effectiveness of these molecular (MAS, MAI) technologies and conventional pure breeding technologies need to be compared. Despite the availability of highly saturated genetic maps for important farm animals (cattle, swine, sheep and chickens), which should provide the genetic framework for developing MAS programmes, records of successes in animals are scarce (Dekkers, 2004). Meanwhile, conventional technologies have proved very effective in the genetic improvement of livestock and, considering the limitations imposed by the biology of the species, large extra gains are unlikely in quantitative traits by using these DNA-based technologies (Perera and Makkar, 2003). The same may, however, not be said about traits controlled by single genes with major phenotypic effects such as the Booroola affecting fecundity in sheep, myostatin for double muscling in cattle and the nematode resistance gene of the Red Masaai breed, where marker assisted introgression offers possibilities for major leaps in genetic gains. The absence of complete phenotypic and pedigree information make it difficult to realize the value of marker information and to determine linkage phase in the case of using linked markers (Perera and Makkar, 2003). Country Reports indicate that several developing nations have no national breeding programmes in place for any farmed livestock species and therefore have not benefited from the conventional tools used for the genetic improvement of livestock. Individual identification and phenotypic characterization of farmed animal breeds to determine their roles in appropriately designed improvement programmes for native breeds would enhance their utilization potential.

4.5 Genotype verification

The growing consumer concerns about the quality of animal products have necessitated the accelerated development of more sophisticated means of tracking livestock products in the food chain back to the farm of origin, a practice referred to as traceability (Souza-Monteiro and Caswell, 2004). The current surge of interest of many meat exporting countries in traceability has been spurred on chiefly by the European Union's beef import requirements instituted in the wake of the bovine spongiform encephalopathy (BSE) crisis. Traceability from farm-to-fork as presently envisaged comprises two stages: the identification of the animal from the farm to the abattoir, and from the cut up carcass to the consumer's plate.

Accurate and secure individual animal identification is vital for several purposes:

- Disease monitoring and eradication programmes
- Control of animal theft within and across borders of countries
- Farm support payment schemes
- To meet requirements of importing countries for traceability.
- Parentage testing in pedigree programmes
- Performance recording in breed improvement programmes

The methods used for individual animal identification has been reviewed recently (ICAR, 2004). Under Title 1 of regulation (EC) 1760/2000 a mandatory system of animal identification for bovines is detailed using two individual ear tags and an animal passport[1]. The DNA identification technology offers a powerful means of authenticating and controlling conventional identification systems. Cunningham and Meghen (2001) have described a number of ways in which DNA technology can be used for live animal identification to authenticate conventional methods. The basis of the methods is the comparison of a query sample with an archived sample previously

[1] An animal passport is a document issued for each animal within 14 days of birth where several records of animal health, movements and production processes are registered.

taken from the same animal at an early age or as an adult. A similar DNA-based, cost-effective identification system for meat has been designed for use in supermarkets (Cunningham and Meghen, 2001). The molecular marker of choice used in genotype verification is the microsatellite. It is now possible using PCR amplification to detect the presence of 211 bp fragments of DNA in meat samples (Jennings *et al*., 2003), thus facilitating the archiving and testing of very small samples of meat.

Meat exporting countries are at different stages of implementing traceability. According to Country Reports, Botswana has just started a national Livestock Identification and Traceability System, which is expected to allow traceability of beef from the farm to the supermarket in compliance with European Union import requirements. The design of the animal identification and recording system for Malawi has now been finalized (FAO, 2004b). This will also be augmented with DNA identification based on archived hair samples as final indisputable proof of the identity of a particular animal. Namibia has rejected branding because it is often not readable when animals grow winter hair coats (FAO, 2004b). Rather, animal identification using ear tags is complemented with paper records, to document the movements of identified animals up to the abattoir. The Brazilian cattle and buffalo herds are expected to be completely identified by the end of 2007. However, traceability along the food chain is only required for producers and processors exporting premium beef, and generally ends at the abattoir (Souza-Monteiro and Caswell, 2004). The Argentinean system has a limited mandatory traceability requirement directed at export markets and covers individual animal identification from the farm to the port where carcasses or beef cuts leave the country (Souza-Monteiro and Caswell, 2004).

Policy issues concern measures to ensure sustainable access to export markets for beef and other animal products as a means of promoting local animal agriculture. An important consideration would be to implement individual identification systems which are accurate and of high precision.

4.6 Genetically Modified Organisms

Conventional breeding tools have proved effective in genetic improvement of several economically important traits in mammalian and avian species. Yet it is still desirable 1) to increase the rate of progress of 1–3 percent currently attainable especially in breeds with long generation intervals and which have never undergone genetic selection. 2) to reduce the number of generations required to separate desired traits from non-desired and detrimental ones in schemes involving more than one breed 3) to undertake a targeted transfer of genetic information between different species, which is now possible in plants but has not been an option for animals and 4) to overcome difficulties associated with the adaptation of introduced breeds to local conditions. Genetic modification of organisms utilizes genes rather than whole genomes. Consumer acceptability of genetically modified animals for food is currently a very contentious issue, even though for medical purposes the expectations are very high for these same modified animals. Two main methods are used to modify the genomes of animals for enhanced agricultural or medical purposes. These are transgenesis and gene targeting /knockout.

4.6.1. Transgenesis

The term transgenic refers to organisms which carry in their genome or express *in vitro*-manipulated gene constructs (Palmiter and Brinster, 1985). Genetic engineering offers the possibility to isolate genes, modify them if needed and reinsert them back into the same or another animal. The production of transgenic animals will lead to a drastic change in the genetic improvement of livestock, since without cross-breeding new genes/traits can be introduced into farm animals. Although today no transgenic animal is on the market, many laboratories are working on them.

To generate lines of genetically modified animals, DNA must be introduced into cells that can transmit the foreign gene to progeny such as one-cell embryos, sperm, oocyte, blastomere and stem cells. Several techniques have been used to transfer genes, depending upon the reproductive system of the species. These have been summarized by Houdebine (2003). DNA **microinjection** is the most commonly used method and consists of injecting purified gene into the pronuclei of fertilized eggs or cytoplasm (non-mammalian species). In pronuclear microinjection, a fine needle is used to pierce the pronucleus and the DNA is injected. The efficiency of this method is 1–3 percent in mice but is negligible (1 in 10,000) in cattle. **Transposons** are natural DNA sequences present in the genome of most mammals with the ability to auto replicate and integrate in multiple sites. The coding sequences of transposons may be deleted and replaced by a foreign gene. Transposons however, cannot harbour more than 5–8 kb of foreign DNA and care must be taken to prevent uncontrollable spread in the genome. Specially designed transposons are available for gene transfer in different species. Various **retroviral vectors** are extensively used successfully to generate transgenic animals including poultry (Mizuarai *et al*., 2001). An improvement of retroviral vectors comes from the use of **lentiviral vectors**. These contain a protein which allows the viral genome to reach chromatin even in quiescent cells, as opposed to conventional retroviral vectors, which can transfer their genes only in dividing cells. Further advantages of lentiviral vectors are their requirement for less resources and simplicity of delivery compared with conventional pronuclear injection (Whitelaw, 2003). A lentiviral vector containing the VSV-G envelope was used to generate a transgenic chicken with a high yield and the transferred marker gene was expressed (McGrew *et al*., 2003).

4.6.2. Gene targeting and knockout

Gene targeting is the definition used for genetic manipulation of animal genomes using homologous recombination. Homologous recombination technique allows the precise modification (replacement or deletion) of determined sequences of the genome. Eliminating a gene (gene knock-out) completely from a diploid organism requires knocking out both copies of the gene in the cells. Deleting gene function is the opposite of transgenesis. Recent achievements include the generation of knock-out pigs for the α-1,3 galactosyl transferase gene, leading to the prevention of hyper-acute immune-rejection of transplanted tissues and organs. The application of gene targeting technologies in domestic animals for xenotransplantation has made significant progress in recent years. Both alleles of α-1, 3-galactosyltransferase gene have been inactivated, which resulted in inactivation of the gene's function (Phelps *et al*., 2003). A further area of application is with prion diseases by knock-out of the PrP gene (Denning *et al.*, 2001). Animal prion diseases include scrapie of sheep and goats, and BSE in cattle. It is expected that removal of the PrP gene from cattle and sheep will result in these animals being resistant to BSE and scrapie. Further evaluations are needed to determine the survivability of $PrP^{0/0}$ domestic animals and their resistance to the diseases.

It appears evident that both infrastructural and human capacity for research in molecular technologies is far more advanced for plant than for animal agriculture in several developing nations such as Ghana, Cameroon, Mali, Burkina Faso, Nigeria (Alhassan, 2003) Brazil, India, China, Argentina and Mexico. Molecular characterisation capabilities exist for livestock work in some developing countries largely with help from international funding agencies, whilst a wide gap exists between nations in the capacity for advanced biotechnologies (Alhassan, 2003).

Biosafety regulations and the efficient testing of GM animals that are likely to be marketed, in order to avoid contaminating the environment, are significant policy issues. This is because the costs involved in the achievement of these objectives preclude the participation of less endowed countries in the use of GM technology. Potential benefits in the areas of disease resistance, adaptability and product quality appear great but have yet to be realized. A lot of work though

needs to be done in more quantitative areas such as growth. These, however, would not be realized in many nations in the next ten years.

4.7 Main applications of transgenesis in animal production.

The main reasons why there is no transgenic animal on any commercial farm yet are:

- in the beginning, key laboratories preferred to work on medical projects where funding was more easily available (Houdebine, 2003);
- limited reproductive ability of animals compared to plants;
- most traits of interest have polygenic inheritance and are difficult to manipulate using transgenesis;
- high cost of procedures relative to value of the product, with less than 2 percent efficiency in farm animals – e.g. 277 attempts yielded only one live sheep, Dolly (Edwards *et al*., 2003);
- anticipated poor consumer acceptance and the related costs of risk assessment of the effects of transgenic animals on herds, environment and consumers will further increase the cost of this technology.

4.7.1. DNA-related technologies in the pipeline

Among the most promising technologies it is worth mentioning:

- Sows expressing bovine α-lactalbumin gene, for more milk, more and heavier piglets weaned (Wheeler, 2003).
- Goats expressing lipid desaturase in their milk have lower levels of saturated fatty acids. Moreover, the milk containing human lysozyme is resistant to spontaneous bacterial infection (replacing UHT sterilization), and would protect consumers against bacterial infections (Murray, 2003).
- Pigs expressing *E. coli* phytase gene in the saliva release 75 percent less phosphate into the environment (Golovan *et al*., 2001)
- The lysostaphin gene prevents growth of *S. aureus* and mastitis in cows expressing it and is expected to save US$ 2 billion/year in the United States alone (Kerr *et al*., 2001).
- Transgenic removal of beta-lacto globulin protein from cows' milk. The protein causes allergies in 10 percent of consumers.
- Transgenic cows overexpressing β- and κ-casein genes have milk enriched in protein and are expected to improve dairy techniques to prepare cheese (Brophy *et al*., 2003).
- A modest effect of transgenesis on growth and carcass quality has been observed in pigs but not consistently in the sheep. The general conclusion of this research is that cost efficiency in relation to the detrimental consequences cannot be justified at present (Besenfelder *et al*., 1998; Houdebine, 2003).
- Despite consistent efforts, attempts to enhance wool growth and wool composition in sheep by transgenesis have given only disappointing results so far (Houdebine, 2003).
- Lactose content in milk has been reduced by secretion of foreign lactase in transgenic mice. This study could be extended to farm animals to reduce consumer intolerance to lactose (Jost *et al*., 1999).

- Pigs carrying human tPA (tissue plasminogen activator) gene have been farrowed in South Korea. The product of the gene prevents blood clots and can be used to treat thrombosis in humans. (FASS, 2003).
- Transgenic chickens have been produced that can lay human protein in their eggs. If extraction methods become commercially feasible, it is estimated that 200 000 transgenic White Leghorn chickens can produce the world's annual supply of insulin in a single day for only a thousandth of the current cost. (FASS, 2003).
- The malarial merozoite surface proteins (MSP) based vaccinations have proved effective in reducing parasite load and raising immune response in humans. Transgenic goats that secrete MSP-1 in their milk are being developed by GTC Biotherapeutics, Massachusetts, United States. (FASS, 2003).

4.7.2. DNA-related technologies on the shelves

Chymogen® (Developed by Genencor International and Marketed by Chr. Hansen's) – Chymogen is the biotechnology-produced version of an enzyme (chymosin) found in calves that makes milk curdle to produce cheese. Because it is produced through biotechnology, it is purer, is more plentiful, and eliminates variability in the quality and availability of the enzyme in calves' stomachs. It is used in approximately 60 percent of all hard cheese products made today.

Posilac® Bovine Somatotropin, Recombinant Bovine Somatotropin (rBST) (Developed by Monsanto) – BST is a naturally occurring protein hormone in cows that induces them to produce milk. rBST improves milk production by as much as 10 to 15 percent and is now used by farmers whose herds represent over 30 percent of the cows in the United States of America. It was approved by the Food and Drug Administration (FDA) in 1993.

4.7.3. Other potential uses of transgenic animals

Generating animals resistant to diseases might reduce the use of antibiotics and other pharmaceuticals, reduce production costs and curb consumer concerns about the consequences of drug residue in animal products. Several diseases are of major interest in this context. Anthelmintics are used routinely in ruminant animals worldwide, whereas the scourge of diseases like trypanosomiasis and swine fever are currently major causes of expenditure in several nations in Africa. The most realistic use of the transgenic procedure will continue to be in the area of medical science where animals are being used successfully as bioreactors because economic returns justify the investment.

4.8 Biosafety and Animal Genetic Resources

Even though no genetically modified food animal exists on any commercial agricultural enterprise, biosafety issues have already generated controversy in the public arena. Generally, people are less comfortable with biotechnology in animals than in plants, perhaps because it involves more complex ethical issues. In a global survey carried out by Environics International (2000), the majority of respondents opposed the genetic modification of animals to increase productivity. In a survey of some West and Central African countries, it was observed that the growing awareness of biotechnology at governmental level has been related to biosafety rather than to the use of the tools of biotechnology to produce tangible products (Alhassan, 2003). All countries in the subregion had taken varying actions ranging from constituting biosafety drafting committees to bringing their biosafety framework documents to the point of legislation.

Biosafety issues arise out of concerns related to (a) the integration of viral and microbial genomes into genomes of animals (see also section 6.7 below) (b) the possibility that transgenic animals

could escape into the environment with consequent impact on wild animal relatives and ecosystems. Besides negative effects of transgenics in the environment, positive impacts are also likely through the use of less chemicals and antimicrobials in the management of disease resistant GM animals. However, the extreme intensification of agriculture that is expected when using highly productive transgenic animals would have implications for animal welfare and environmental pollution. Biosafety regulations would receive greater attention than at present, when it becomes possible to use transgenic animals for food. The present use for pharmaceutical and medical purposes raises fewer concerns because of the lower risk of unwanted release into the environment.

5 DNA-related technologies and animal nutrition

Feed constitutes 50 to 75 percent of the total production costs of many livestock enterprises. Major efforts in making livestock products affordable in developing nations often target reducing feed costs. Finding better ways of using the vast abundance of fibrous biomass inedible to humans would dramatically improve the prospects of ruminant production in several tropical developing regions of the world. Recent advances in our understanding of the enzymes that modify cell wall architecture, the cloning of the first cellulose synthase gene and revisions to the lignin biosynthetic pathways have facilitated the development of new strategies to alter cell wall properties in transgenic plants (Chapple and Carpita, 1998). It appears apparent that application of gene-based technologies to improve utilization of fibrous feeds are well advanced in plant breeding compared with animal approaches, which are technically more difficult, have longer generation intervals and face more complex ethical issues.

Several areas of research in plant breeding (McSweeney and Makkara, 2003) are focused on 1) reducing the concentration of compounds that retard digestion (e.g. tannins, lignin and toxins) 2) optimising the concentration of desirable compounds (e.g. non-degradable proteins, sulphur amino acids, and soluble carbohydrates) and 3) genomics of rumen micro-organisms especially fibre degraders. Capacity should be built through North-South bilateral collaboration to enable plant breeders and microbial geneticists in developing nations to participate in these studies in step with developments in developed nations based upon the conditions in their own countries (forages etc.).

Genetically-modified maize and soya for herbicide and insect resistance are expected to lower production costs of these ingredients of livestock feed. In those developing countries where small-scale, labour-intensive crop production systems prevail, the gain may, however, be lower compared with large-scale commercial monocropping systems. Genetically modified maize with enhanced amino acid content and which also reduces phosphorus and nitrogen excretion in swine and poultry will improve feed efficiency and minimize the negative impact of non-ruminant production systems on the environment. Estimates for 1999 indicate that 39.9 million hectares of land were planted with transgenic crops (FAO, 2001) mainly in Argentina (18 percent) and the United States and Canada (72 percent). By 2003, the number of countries that had adopted GM crops and the acreages cropped had risen to nine and 68 million hectares respectively (Eveson, 2004). These GM products are, thus, ready for use barring consumer acceptance in developing nations.

6 Concerns about food safety risks of transgenic animals and their feed

The food safety risk of GM animals has been the subject of discussion of many expert meetings and reviews, the latest being FAO (2004b). The concerns are related to the methods applied in the transfer of genes to crops and animals. Two stages are involved in the genetic modification of crops and animals: 1) one of the methods of screening for recombinant DNA molecules cloned into a vector (e.g. plasmids) involves the use of genes coding for antibiotic resistance as markers; 2) insertion into the genome occasionally involves the use of retroviral vectors, and transposons (Houdebine, 2003).

Viral-based technologies take advantage of the ability of viruses to "cut into" the sequences of host DNA. Such interruptions may be benign or hazardous. Food safety issues which arise from this technology are in relation to the infectivity of the vector, the assessment of potential effects of vector regulatory elements on the host cell and the possibility of recombination with endogenous viral sequences (Kok and Jones, 2003). Transposon-based technologies have also been developed. Transposons are often referred to as "jumping genes" because of their unique ability to catalyse their own movement within the genome of the animal.

In consideration of the methods of transformation outlined above, the minimal food safety assessment of GM animals acceptable to a wide range of experts comprises (Kleter and Kuiper, 2002): 1) the molecular characterization of the inserted foreign DNA; 2) the safety assessment of the introduced genes and their products; 3) assessment of any unintended effects of the insertion of foreign DNA in the organism; and 4) assessment of the effect of disease-causing agents.

Fears expressed concerning the use of genetically modified crops to feed animals include:

- whether modified DNA from the plant may be transferred into the food chain with harmful consequences;
- whether antibiotic resistance marker genes used in the transformation process may be transmitted to bacteria in the animal and, hence, potentially into human pathogenic bacteria;
- possible accumulation of transgenic DNA and protein in milk, meat and eggs derived from animals receiving GM ingredients.

Researchers have shown that processed feed samples may contain DNA fragments large enough to contain functional genes and, thus, livestock will consume both transgenic DNA and protein (Phipps, Beever & Einspanier, 2003). However to date, neither transgenic DNA nor recombinant gene products have been detected in milk, meat or eggs derived from animals receiving GM feed ingredients as part of their diet (Phipps, Beever & Einspanier, 2003). Attempts to detect transgenic and endogenous plant DNA and transgenic protein in the breast muscle of broilers fed YieldGard® Corn Borer Corn gave negative results (Jennings *et al.*, 2003). In spite of these results, Chambers and Heritage (2004) after extensive review concluded that markers that code for resistance to clinically significant antibiotics critical for treating human diseases should not be used in the production of transgenic plants.

7 Conclusions

1. An attempt has been made in this paper to use literature particularly that published after 1999 to review modern biological technologies that may be applied in the management of animal genetic resources. Cunningham (1999) reviewed pertinent literature up to 1999. Country Reports give insights to the state of capacity of nations as regards management of animal genetic resources (AnGR).
2. Demand for livestock products in developing countries has been predicted to continue to grow faster than internal production, resulting in trade deficits. This growing demand offers an opportunity for income generation for the rural poor who depend greatly upon livestock for their livelihoods. However, the productivity of native breeds needs to be improved to enhance their utilization and income generating potentials, as well as to safeguard against their marginalization and future extinction.
3. The use of AI appears warranted within the context of an ongoing, appropriately designed breeding programmes encompassing the national herd, which would be structured and supplemented with individual identification and performance recording at the farm level. National improvement programmes with links to regional programmes for the same breeds have several potential benefits. These would facilitate the shared use of several facilities such as AI centres and cryobanks.
4. Using sexed semen and embryos, it is possible to predetermine and in fact customize the sex ratio of the progeny. The reduction in the number of animals in the herd possible with this technology would impact positively on the environment. Sexed semen is commercially available in some developed countries such as the United Kingdom and with further reductions in production costs, should become affordable in developing nations in the not too distant future.
5. The use of embryo transfer (ET) technology is still too expensive to be used routinely for animal production in many developing countries.
6. *In vitro* maturation and fertilization would further extend the use of cows of high genetic merit. When applied to young female animals, it can accelerate genetic progress by increasing selection intensity and reducing generation intervals. The cost of the technology limits its widespread use for food animal production.
7. The successful adoption of these mature (ET and AI) reproductive technologies is predicated upon the premise that market trends are favourable. Both technologies are widely used in breed development in the dairy industry where demand for milk products justifies their adoption. Dairy development initiatives in some developing countries have been stifled by the absence of supply contracts for liquid milk. Thus, the global trade policy environment plays a crucial role in influencing the adoption of modern biological technologies to improve animal agriculture.
8. Progress has been made in the cryopreservation of chicken semen with fertility rates post-thawing occasionally reaching 90 percent. This has implications for *ex-situ* conservation of chickens in the near future. Embryos are the best choice if the purpose is to restore a desired genotype since, unlike semen, embryos represent diploid genotypes. It is becoming increasingly possible to cryopreserve the embryos of sheep, goats and pigs in addition to cattle. However, success rates (number of offspring per frozen embryo) with pigs are still very low (10–20 percent). On account of cost considerations, the establishment of regional genebanks has several benefits. However, the legal framework to regulate their operation must be carefully drafted.

9. Cloning has promise for the dissemination of genetic improvement, and the generation of uniform end products to meet the demands of a traditionally stable market. It may also be used to generate "copies" of highly adapted genotypes from well designed improvement programmes in low input systems. Even though subsequent improvement in the performance of clones as well as the flexibility to respond to changing market demands have been questioned, genetic variability could still be retained by using cloned sires on a random sample of females. The current costs, resulting from technical inefficiencies, make it inaccessible for animal agriculture. Dolly was the only outcome after 277 attempts. Further the death of Dolly raises concerns about viability of clones.
10. The use of neutral markers such as microsatellites for measurement of genetic diversity has benefits when making decisions for breed conservation and utilization.
11. Despite the availability of dense genome maps, the use of MAS has yet to make an impact in animal production. Marker assisted introgression of specific genes from one breed into the other in combination with genomic selection offers the possibility for rapid genetic leaps. For developing countries, introgressing genes for adaptation into more productive genomic backgrounds could be a fast track approach to breed improvement. There is however, paucity of literature in this regard.
12. The era of automated DNA sequencing and genome databases (genomics) has arrived and with it have come new methods for identifying particular genes and their protein products (proteomics). It is the expression of a set of genes in a particular cell type which defines the function of that cell. The pattern of gene expression can change during development or as a result of disease. Research into gene expression and proteomics will enable scientists to decipher the functions of genes and their protein products, and to get a clearer picture of the complex regulatory networks that control fundamental biological processes.
13. DNA identification technology is worth consideration by developing nations with the potential to export animal products. It offers a powerful means of authenticating and controlling conventional identification systems. As consumers become increasingly health conscious, the demands for traceability from the supermarket to the farm of origin, now being enforced by the EU, will soon become a global phenomenon as all importing countries will require it.
14. The most realistic use of the transgenic procedure will continue to be in the area of medical science where animals are being used successfully as bioreactors, because economic returns justify the investment. Its use in most developing countries cannot be foreseen in the next half decade on account of a lack of expertise and funds for capacity building.
15. Although slow, progress is being made towards the generation of transgenic animals for food and agriculture. An important event in the future would be the capacity to target gene integration in the host genome (Houdebine, 2003) to avoid insertional mutagenesis in animals.
16. Public acceptability of biotechnologically modified animals for food will determine to a large extent future impetus and support for research work in this direction. Issues related to ethics and biosafety regulations appear to be more important than tangible products of such animals for food. Several developing nations have never benefited from current methods of genetic improvement of farm animals. An important consideration concerns steps to implement such schemes by instituting individual identification and recording

schemes and adoption of appropriate pure-breeding programmes that do not dilute genetic biodiversity.

17. For the foreseeable future (one to five years), developing countries are not developing gene-based technologies. However policy issues concerning their adoption need to be addressed. This requires the development of capacity in terms of skilled human resources and technical wherewithal to test for the suitability of gene-based products for the local production environments as well as health risks to consumers.The adoption of transgenic technology for animal production by developing nations, at best, would be of research interests only in the near future (five to ten years) and even this would depend upon substantial infusion of capital and technology transfer through bilateral North–South technical cooperation.

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