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A Vital Role of Biotechnological Tools in Bovine Production

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ABSTRACT
Biotechnology in livestock production has emerged as a tool for improving nutrition, animal health, genetics, reproduction, and production to fulfill the increasing demand for food from livestock products due to population and income growth.

***Keywords**: Biotechnological tools, bovine production, livestock.*

INTRODUCTION
Since 1953 when Watson and Crick published their work on the structure of DNA, opportunities to apply molecular biology techniques in medical, veterinary and agricultural fields have increased at a remarkable rate. The comprehensive name given to these developments has been biotechnology. Biotechnology is defined as the use of living organisms, cells, sub-cellular organelles, and/or parts of those structures, as well as the molecules to effect physical or chemical changes needed to generate new products for research and commercialization . The principal application of animal biotechnology at present is in the production of cheap[1] and dependable diagnostic kits and vaccines. Innovations in diagnostics and veterinary medicines have played an important role in increasing the animal productivity. Genetic manipulation is increasingly being used in livestock industry to identify and select the particular genes that lead to these desirable traits and it is now possible to select superior germplasm and disseminate it using artificial insemination, embryo transfer and other assisted reproductive technologies. These technologies have been used in the genetic improvement of livestock, particularly in cattle and buffaloes, and the economic returns are significant. Gene mapping and marker assisted selection may enable new genetic variation to be introgressed into one breed from another of the same species. Marker assisted selection has the potential to increase accuracy of selection and also reduce the generation interval, especially if used in conjunction with reproductive technologies that are under development. Gene mapping may also allow the identification and isolation of genes that may be fed back into genetic manipulation programmes [1].

Major fields of Biotechnology in Animal Production [2]
The major fields in animal biotechnology include:
1) Manipulation of reproductive processes
2) Genetic engineering of macro-organisms
3) Genetic engineering of micro-organisms and molecules including cell-engineering
   - ✓ hybridomatechnology to produce desired end products such as vaccines, gene probes,
   - ✓ monoclonal antibodies and growth promoters.

Reproductive Technologies Process
a) Artificial Insemination, Estrus Induction, Super Ovulation
Manipulation of reproductive processes in domestic species started in the 1936s with Artificial Insemination (AI). Its use became widespread in the 1960s when AI organizations began to make routine use of frozen semen. Manipulation of female reproductive processes

started with oestrus induction and synchronization, initially with steroid hormones and later with prostaglandins. Super ovulation technique with gonadotrophic hormones led to embryo transfer on an increasing scale in the 1980s when embryo freezing techniques became available. AI and embryo transfer techniques offer animal breeders a number of opportunities to enhance the rate of genetic progress in national breeding programmes.

b) Embryo Transfer

Although not economically feasible for commercial use on small farms at present, embryo technology can greatly contribute to research and genetic improvement in local breeds. As a method, ET basically requires synchronization of the donor and the recipient females so that the embryos are recovered and transferred in synchrony in order to warrant a proper embryo elongation and the recognition of pregnancy by the recipient cow. ET does not have be done immediately, and bovine embryos can be frozen, either conventionally (slow freezing using ethylene-glycol) or by vitrification (high concentrations of cryoprotectants and plunging into LN2), ensuring safe storage and better management of the genetic resources. The principal benefit of embryo transfer is the possibility to produce several progenies from a female, just as AI can produce many offspring from one male. For example, the average lifetime production of a cow can be increased from 4 to 25 calves. Increasing the reproductive rate of selected females has the following benefits: genetically outstanding animals can contribute more to the breeding programme, particularly if their sons are being selected for use in AI; the rate of genetic change can be enhanced with specially designed breeding schemes which take advantage of increased intensity of female selection combined with increased generation turnover; transport of embryos is much cheaper than that of live animals; risk of importing diseases is avoided; facilitates rapid expansion of rare but economically important genetic stocks; and the stress to exotic genotypes can be avoided by having them born to dams of local breeds rather than importing them as live animals [3].

c) *In vitro* Embryo Production, Embryo Sexing, Embryo Splitting, Embryo Cloning

More advanced techniques for altering reproduction in livestock have been developed in the last decade. Methods for in vitro maturation (IVM), fertilization (IVF) and culture (IVC) are available for cattle, proved by the birth of innumerable calves worldwide. These include oocyte removal from genetically superior cows during the dioestrous period, in vitro maturation and fertilization of these oocytes, in vitro cultures of fertilized oocytes (zygotes) up to the blastocyst stage, sexing, splitting and cloning of embryos. Splitting and cloning of sheep and bovine embryos became possible after micromanipulation techniques were developed [4].

d) Sexing of Speramatozoa

Sexing spermatozoa for directed production of offspring of a desirable sex by use of modified flow cytometric cell sorting of fluorescent dye loaded living spermatozoa is an important biotechnological intervention. Cattle present about 3.8% differences in DNA contents between their X- and Y-chromosome bearing spermatozoa, a difference large enough to allow successful sorting. Although the numbers of sorted spermatozoa per hour reach at present larger figures that for a decade ago (50-100 million compared to 350,000), these number simply few sperm doses for AI, impairing their application for conventional breeding. The technology is, however, very promising and provides opportunities for sex-selection in livestock. The process of commercialization of sexed sperm has accelerated recently. However, this technology is characterized by high costs, complexity of implementation and lower pregnancy rates than with control sperm [4].

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Genetic Technologies

Transgenics

Transgenics provide methods to introduce, rapidly, 'new' or modified genes and DNA fragments into livestock without crossbreeding. The development of transgenic farm animals will enable greater flexibility in direct genetic manipulation of livestock. Gene transfer is a beneficial way of modifying the genome of domestic livestock. The use of these methodologies will have a great effect toward improving the efficiency of animal agriculture. Practical applications of transgenics in livestock production include improved milk production and composition, increased growth rate, improved feed usage, improved carcass composition, increased disease resistance, enhanced reproductive performance and increased prolificacy. Additional applications include altered cell and tissue characteristics for biomedical research and manufacturing. Micro manipulation techniques have already been used to produce transgenic mice, sheep, pigs and cows. Gene constructs, coding for a known characteristic, can be injected into the pronucleus of a fertilized ovum. This technique enables the transfer of any gene from any source, no matter how remote the relationship between the donor and recipient is, into a population of animals. The ultimate success rate depends on whether the gene is properly expressed in the recipient and their offspring; *i.e.* whether the protein specified by the introduced gene is synthesized or not. These new technique fulfill the long-range goal of animal breeders which is to introduce particular genes into the germplasm of domestic animals. This may, together with manipulation of reproductive processes, improve disease resistance, milk production, growth, feed conversion efficiency and overall economic merit. Genetic engineering also yields new insights into basic physiological mechanisms (*e.g.* gene regulation), expedites the production of animals with desired production trait sand will result in the creation of animals with entirely novel properties (*e.g.* secretion of biomedical substances in milk), completely unattainable by conventional breeding and selection techniques. However, it must be stated that considerable research is still needed as the present success rate is 0.5-1% only [4].

Marker Assisted Selection or Marker Aided Selection (MAS)

Marker Assisted Selection is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (*i.e.* productivity, disease resistance, abiotic stress tolerance, and/or quality). This process is used in plant and animal breeding. Considerable developments in biotechnology have led plant breeders to develop more efficient selection systems to replace traditional phenotypic-pedigree-based selection systems. Marker assisted selection (MAS) is indirect selection process where a trait of interest is selected not based on itself but on a marker linked to it. For example if MAS is being used to select individuals with a disease, the level of disease is not quantified but rather a marker allele which is linked with disease is used to determine disease presence. The assumption is that linked allele associates with the gene and/or quantitative trait locus (QTL) of interest. MAS can be useful for traits that are difficult to measure, exhibit low heritability, and/or are expressed late in development [5].

Genetic Engineering of Micro-organisms and Molecules, including Engineering of Cells

a) Vaccines

The production of recombinant vaccines against various animal diseases that are caused by bacterial, viral or parasitic infections is a field of active research. Most vaccines used in livestock at present are still produced by conventional methods. At present, an enormous amount of research is going in this area in an effort to develop new, safer and more efficacious vaccines which give the highest protection to the animal economically. There are a large number of animal diseases for which protective vaccines are yet to be available, especially against parasitic diseases. Some antigens have been virtually impossible to incorporate into vaccines because of the difficulty in growing the micro-organisms or isolating the antigens, or because of their complexity or an inability to adapt them to a viable production method. Research activities are now focused on subunit, recombinant DNA, synthetic peptide, anti-idiotype, deletion mutant, re assortment and vaccine vectored vaccines. It is expected that in the next few years an increasing number of genetically engineered vaccines will be marketed [6].

b) Application of DNA/RNA Probes

The emergence of recombinant DNA technology has given rise to diagnostic DNA/RNA probes which are based on the ability of single stranded DNA or RNA to form hybrids with a complementary labeled sequence of nucleotides on another strand of DNA/RNA. This means that DNA/RNA sequences unique to a species can be recognized by hybridizing or pairing test material with DNA, labeled in are cognizable manner. Application of DNA/RNA probes in the diagnosis of infectious diseases is a recent alternative to the established isolation and determination of micro-organisms by cultural and serological methods. Expectations for this new technique are high, because probes may have advantages over the conventional bacteriological and virological methods such as a constant and well-defined sensitivity and specificity which enable identification of pathogens in a relatively short time, and their direct detection in clinical specimens. Other probes and techniques are also becoming, available for the detection of genetic errors, the determination of sex of embryos, the verification of pedigrees and the monitoring of physiological changes induced by the introduction of new genetic material [7].

c) Monoclonal Antibody Technology

Parallel with developments in DNA technology are the advances in immunochemical diagnostic procedures. Serological diagnostic tests, used for many years rely on the detection of antibodies stimulated by invading organisms. The use of antibodies to detect infectious agents, proteins or pharmaceutical substances is exemplified by the expansion of monoclonal antibody technology. This involves the fusion of somatic cells to form a hybrid or hybridoma from both parents. Hybriodoma can produce antibodies (monoclonals)specific for a single antigenic determinant (epitope), which can be produced in large quantities. Monoclonal antibodies, when labeled may be used to detect substances in tissues, fluids, or cells even under field conditions. A practical example is the bovine milk progesterone test for determining the stage of the oestrous cycle. However, many other monoclonal antibodies have been produced against a number of antigens: tumour cell markers, histocompatibility antigens, lymphocyte differentiation antigens, blood group antigens, bacteria, viruses, fungi, protozoa, helminths, hormones, enzymes, nucleic acids, immune globulins, and receptor sites. It is expected that hybridoma technology will deliver more powerful products for both the human and veterinary medical markets in the future [7].

d) Growth Promoters

(i) ***Range of Hormones produced by Genetic Engineering***

Most of the functions of the body are controlled by hormones, chemical substances produced naturally in different glands, which in minute quantities influence the performance of specialized groups of cells. Individually, they are responsible for the functions of growth, reproduction, milk secretion and the metabolism of the body in general. A number of chemical and pharmaceutical companies abroad have genetically engineered hormones or growth promoters such as human (hST), bovine (bST) and procine (pST) somatotropin, interferon, lymphokines etc. These are in the field testing stage and are ready to market for use in human and veterinary medicine. The use of these products may help to correct growth retardation in children, make hogs grow faster with less fat and more lean production of dairy cattle [8].

(ii) ***Biotechnology contributions to Animal Diets***

Other growth promoters such as antibiotics and non-antibiotic tools including enzymes, yeast cultures, live bacteria and their metabolites and feed pH adjusters are being perfected for use in livestock farming. These products should offer the nutritionists, if used appropriately, great potential for improvement in their animal nutrition programmes. Addition of suitable crude enzyme preparations to diets of both pigs and poultry can lead to improvements in feed conversion efficiency and live weight gains.

(iii) Probiotics

Other biological tools are the probiotics which encompasses organisms and substances that contribute to intestinal microbial balance. Applications are mainly in poultry, pigs and calves. The aim is a constant infusion of friendly organisms, such as lactic acid bacteria, via the diet, to prevent colonization of the gastrointestinal tract by disease causing organisms. It is thus based on the principle of competitive exclusion.

Impact of Technologies

There are a large number of technologies that have been developed for or adapted to the livestock of our country. However, the major technologies that are used effectively in livestock production in the developing world include conserving animal genetic resources, augmenting reproduction, embryo transfer (ET) and related technologies, diagnosing disease and controlling and improving nutrient availability also. Animal biotechnology is the result of a multistage process, involving research, development, testing and registration, production and marketing. The goal is to develop a technology, process or product that has clear commercial potential and can be commercialized after due testing and regulatory approval. However, several technologies from developed countries have been successfully adopted by developing countries. Studies from India have shown that technological input is responsible for about 45% of total output growth and that the TFP growth may be as much as 1.8% [8].

Production of Good Quality and High Yielding Animal

Livestock production is expected to grow tremendously in line with the projected demand for animal products. Therefore, the methods of livestock production must be changed to allow for efficiency and improvement in productivity. Biotechnological research is important in order to respond to the pressure of producing more food from animals to cater food requirement of the ever-growing human population. Transgenic animals such as mice, rats, rabbits, pigs, sheep, and cows have been developed with the help of biotechnology. Transgenesis is the technique that permits the manipulation of genes of one organism which can subsequently be introduced into genome of another organism of same or other species in such a way that the genes are not only expressed but also gets transmitted to its progeny [9].

Improvement in Quality of Livestock Products

Major genes for meat quality offer excellent opportunities for increasing level of meat quality and decreasing variability. Most scientists say that tenderness is 30 per cent and Pale Soft Exudate (PSE) condition in swine is 50 per cent genetically governed characters. Gene that affects tenderness of meat before slaughter is CLPG in sheep, myostatin in beef, RN in pork. Identification, isolation and modification of useful genes are some of the important aspects of biotechnology research and development. The quality of carcass can be improved by manipulating the lipoprotein receptor and leptin genes thereby the cholesterol and fat content of meat can be governed.

Production of Hormones

There is a growing database supporting the use of pituitary derived somatotropin (ST) as an agent to improve growth and carcass composition. Pigs injected with pST had 35 per cent less fat and 8 per cent more protein. However, purification of ST from Pituitary gland is uneconomical as production of single dose requires collection and processing of 25-100 Pituitary glands. More recently, the development of recombinant DNA technology has provided a mechanism for large scale production of somatotropin. The gene for ST protein is inserted in laboratory strain of *E. coli* which can be grown on a large scale and from which ST can be purified and concentrated. Bovine and ovine ST improves growth rate by 20 per cent and lean to fat ratio by 40 per cent in ruminants. While experimentation in growing pigs, significant improvement of 40 per cent in average daily gain and 30 per cent in feed conversion efficiency had been achieved by administration of pST. Furthermore 60 per cent reduction in carcass fat and 70 per cent increase in carcass protein had been attained. No significant difference between effectiveness of pST and rpST had been observed [10].

Production of Enzymes

Dairy industry requires large amount of rennet to produce cheese in bulk. Traditionally rennet is procured from calves. However due to global imbalance between production of cheese and calf slaughter and worldwide shortage of calf rennet, exploration of alternative source of rennet is required to be investigated. In certain countries like India, religious feeling has aggravated the need to rennet substitute [11].

Treatment of milk with galactosidase results in hydrolysis of lactose, thereby making it digestible by lactose intolerant people. The enzyme galactosidase hydrolyses lactose into glucose and galactose. Since these enzymes are costly, biotechnology can help in its economic production as well application. Commercial -galactosidase is produced from yeasts such as *Kluyveromyces lactis* and *Kluyveromyces marxianus* (formerly known as *Kluyveromyces fragilis* and *Saccharomyces fragilis*), and moulds such as *Aspergillus niger* and *Aspergillus oryzae [10].*

Functional and Designer Livestock Products

In order to improve the product, attempts can be made to develop strains of starter cultures capable of enhanced anti-chlolestermic attributes, enhanced anti- carcinogenic attributes, enhanced antagonistic influence on enteropathogenic microorganism. The genetic stability of starter strains, bacteriophage infection, production of off-flavour and insufficient development of acid during fermentation are costly problems to dairy industry. Hence there is a great deal of interest in the development of new and improved strains, using modern techniques of molecular biology viz. plasmid transfer, transduction, protoplast fusion and cloning. Plasmid biology of lactic acid bacteria have opened new vistas for exploring possibilities for using recombinant DNA technology and genetic engineering to improve the nutritional or therapeutic value of these products. A complete phage resistant strain can be achieved by incorporating plasmids which are inhibitory for phage absorption, penetration and DNA metabolism. Genetically engineered strains can play a vital role in manufacturing tailor made high quality fermented livestock products. Cloning of genes from lactic acid bacteria could be carried out in food grade strains of *E. coli* for which vectors & transformation systems are available [8].

Bio-preservation

Livestock products being highly prone to microbial contamination and they undergo microbial deterioration. The recent trend of consumers towards food without artificial preservatives has led processors to search for natural preservatives. The antimicrobial system possessed by lactic acid bacteria offer scope for the development of an effective natural preservation process for application in food either as purified chemical agents or as viable cultures.Suppression of spoilage and food borne pathogens by lactic acid bacteria could be extremely beneficial to human health and dairy industry as these attributes can considerably improve the shelf life and safety of fermented foods. Lactic acid bacteria can act antagonistically against a wide range of food borne pathogens and spoilage organisms like *Salmonella*, *Staphylococcus, Clostridium, Listeria monocytogenes, Yersinia enterocolitica and Psuedomonas*. Incorporation of certain antimicrobial agents to livestock products viz bacteriocin, nisin, H_2O_2, diacetyl, microgard, reutrin and pimaricin has yielded highly promising results and the enhanced shelf life of products thus attained would be economically, nutritionally and therapeutically beneficial both to manufactures & consumers. Biotechnological techniques can now be applied to develop strains of lactic acid bacteria capable of enhanced production of these natural food grade preservative and also to combine within a single strain the ability of produce a number of such bacteriocin to extend their antibacterial spectrum [8].

Byproduct Utilization

A major concern in the food processing industry is the development of methods to convert inedible and waste materials into new value-added products. Environmental and economic concerns necessitate a reduction of food processing waste, better use of raw materials and by products to new value-added products. The cheese industry generates billions of pounds of whey that must be disposed off. Ultra-filtration has provided the cheese maker with a means of concentrating the protein component of whey into a value-added item with significant dollar value. A recently developed bioconversion system employing selected strains of yeast can convert these solids to ascorbic acid with a market value of about $10 per kilogram. The yeast biomass could be dried and used as single-cell protein supplements in animal feed. Enzymatic treatment of food processing waste streams can produce materials readily metabolized by genetically engineered micro-organisms to produce antibiotics, hormones or peptides of interest in the pharmaceutical or chemical industries [8-9].

Quality Control

Ensuring an acceptable level of food quality and safety is absolutely necessary to provide adequate protection for consumers and to facilitate trade. There must be a strong network of efficient quality assurance programme to monitor the quality and safety of these foods before reaching to the consumers. This can be largely possible with the application of recent developments in biotechnological tools in quality assurance programmes. The use of modern biotechnology has proved to be rapid, sensitive and accurate methods for detection and analysis of bacterial contaminants and pathogens or their toxins. Some of the most powerful tools of biotechnology which have already made great strides include rDNA technology, Genetic engineering, PCR, Microarray etc. Random amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) molecular marker systems can also be used for the comparison of genetic differences between species, subspecies and strains, depending on the reaction conditions used. The use of combination of these technologies and other genetic tests allows the characterization and identification of organisms at the genus, species, sub-species and even strain levels, thereby making it possible to pinpoint sources of food contamination, to trace microorganisms throughout the food chain or to identify the causal agents of food borne illnesses. Microarrays are biosensors which consist of large numbers of parallel hybrid receptors (DNA, proteins, oligonucleotides). Microarrays are also referred to as biochip, DNA chip, DNA microarray or gene arrays and offer unprecedented opportunities and approaches to diagnostic and detection methods. They can be used for the detection of pathogens, pesticides and toxins and offer considerable potential for monitoring the quality and safety of raw materials. Biotechnology has led to the development of tests that can be completed in one fifth of the time required by conventional methods. Development of new innovative methods for rapid detection of emerging high risk food pathogens such as *E. coli O157*, *Listeria monocytogenes*, *Salmonella* and *Yersinia enterocolitica* in food of animal origin is extremely important in context of food safety [9].

Meat Verification

In food production, raw materials from farm animals are typically processed to meat and cheese products. Efficient assays to detect the origin of meat precisely and rapidly are always in demand. Identification of the meat origin from processed meat products is a current matter of concern for a variety of economic, religious, and health reasons. The extensive development of nucleic acid based technologies over the past decade reflects their importance in food analysis. DNA analytical methods offer a promising alternative for reliable species differentiation even in heated food samples, since the thermostability of DNA is much higher than that of proteins. Most of the DNA-based methods used rely on the simplicity and the sensitivity of the polymerase chain reaction (PCR). Species differentiation is achieved either by amplification of characteristic DNA fragments with species-specific primers or in the consensus PCR approach, by use of universal primers followed by sequencing or restriction fragment length polymorphism (RFLP) analysis for the species-specific identification of the resulting amplicons [9].

Adoption and Adaptation of Biotechnology in Field

The case of artificial insemination **(**AI) is a good example, Where AI technology has been every well adopted in India. Whereas, in case of Embryo Transfer (ET), could have a major impact on cattle breeding especially as part of a nucleus breeding scheme. However, successful ET requires highly motivated, experienced staff and a high capital investment in facilities, equipment and drugs. In general, the inappropriateness of ET for developing countries is ascribed to lack of infrastructure. However, in some instances, ET represents a solution to a lack of infrastructure. Thus, establishment of multiple ovulation embryo transfer (MOET) is considered an attractive means of genetic improvement where infrastructure for progeny testing is not available. We have limited financial resources. Thus, by combining good imagination with knowledge of basic principles, the technology can be successfully adapted to local conditions. Therefore research, especially of an applied nature, on such technologies by institutions in developing countries is always justified and often essential. Moreover, researchers need to be exposed to new technologies or procedures to appreciate the power and limitations of such technologies [11].

LIMITATIONS

The application of new molecular biotechnologies and new breeding strategies to the livestock breeds used in small holder production systems in developing countries is constrained by a number of factors. In the developing world, poverty, malnutrition, disease, poor hygiene and unemployment are widespread, and biotechnologies must be able to be applied in this context [10].

Over the last few decades, the green revolution has brought comparative prosperity to farmers with land, but majority of farmers, who are landless or marginal farmers and subsist only on livestock, have been neglected and remain poor. The major constraints on applying biotechnologies have been enumerated as given below:

1) Absence of an accurate and complete database on livestock
2) Lack of trained scientists, technicians and fieldworkers to develop and apply the technologies, both in the government and in the private sectors.
3) High cost of technological inputs
4) Biodiversity within species and breeds in agro-ecological systems
5) Species and breeds are unique to the developing world; each has its own distinct developmental, production, disease resistance and nutrient utilization characteristics
6) Absence of an interface between industry, universities and institutions, which is necessary to translate technologies into products
7) Inability to access technologies from the developed world at an affordable price in order to make a rightful, positive and sustainable contribution to livestock production and the economic welfare of farmers
8) Failure to address issues of biosafety and risk analyses of new biological, gene products, transgenic and modified food items etc [11].

CONCLUSION

Although animal production is being changed significantly by advances made around the world, benefits are reaching the developing world in only a few areas such as conservation, animal improvement, healthcare (including diagnosis and control of disease) and the augmentation of feed resources. Adopting biotechnology has resulted in distinct benefits in terms of animal improvement and economic returns to the farmers. Over the past decade, the Indian animal science research institutes have focused on biotechnological applications and have multi-institutional programmes to develop and apply biotechnology. Global output of the livestock sector 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. 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.

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