Review Article

The Journal of Animal & Plant Sciences, 23(4): 2013, Page: 944-955
ISSN: 1018-7081

THE FUTURE PROSPECTIVE OF GENOMIC BIOTECHNOLOGY IN ANIMAL BREEDING: THEIR POTENTIAL FOR LIVESTOCK PRODUCTION IN PAKISTAN

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ABSTRACT

This review paper discusses the exciting scientific and technical advances in genomic biotechnologies which covers a wide range of high throughput techniques for the genetic improvement in animal and livestock production. Advances in molecular genetics and in high throughput technologies for genotyping and sequencing now facilitate the integration of molecular data in genetic improvement programs for livestock on a more comprehensive basis. Moreover, these methods enable a change from using markers on a within family basis for detection and selection on quantitative trait loci (QTL) to using markers that are linkage disequilibrium across the population. Biotechnology has been directed primarily towards reproductive technology have been employed for genetic improvement of farm animals which is foremost concern over the years for scientist and researchers. Transgenesis and cloning are expected to have a significant impact on the future genetic improvement of livestock. This review also described the most recently developed technologies such as GeneChip technology and Next generation high throughput DNA sequencing techniques are opening the fascinating opportunities, provides efficient access to genetic information and enables us to detect functional genes and markers of important traits to facilitate molecular breeding. Particularly every developed and developing countries are engaged in molecular biological activities. The productivity in animal agriculture in Pakistan will need to be significantly increased using these recent genomic and biotechnological methods with the intention of satisfy increasing consumer demand, to more efficiently exploit scarce resources and to generate income for growing agriculture population. Interdisciplinary cooperation will also be needed among industries, consumers and research institution.

Key words: Genomic biotechnology, Quantitative trait loci, GeneChip technology, Next generation DNA sequencing, Pakistan

INTRODUCTION

Animal genomics is of interest because of its importance to produce high quality food products economically and efficiently to furnish for the increasing supply demand gap all over the world. There is evidence for a rapidly increasing demand for livestock products in developing countries like Pakistan as a result of high population and income growth and life style changes. Livestock sector has come out as a leading sub-sector of the agriculture of Pakistan. Due to unplanned population growth in Pakistan, there is a terrible need to increase the productivity of dairy animals by the application of modern genetic and breeding technologies such as identifying major genes involved in determining the genetic potential for animals for high milk production, milk protein, fats, growth rate, energy metabolism and diseases resistance.

Genomic biotechnologies in farm animals offer a major opportunity to address shortages in agriculture production to feed the global society at large. Benefiting from the PCR techniques, the Molecular markers have now become a fashionable ways for the identification and characterization of animal species. In the last decades a number of marker techniques were consequently developed, in particular RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), RAPD (randomly amplified polymorphic DNA), microsatellite (simple sequence repeat) and SNP (single nucleotide polymorphism). The presence or absence of markers allows the genotyping of individuals and populations. A very exhilarating and fast developing application of genetic markers is in the mapping of the various animal genomes. The theoretical studies of linkage mapping, finding quantitative trait loci (QTLs) and the marker assisted selection or genotype selection have been developed in the previous decade. DNA markers can be employed to make out the specific region of chromosome where genes affecting quantitative traits are located. One approach is known as Marker assisted selection (MAS) uses information about these sections of chromosomes in livestock selection programs to recognize individuals with favorable combination of

quantitative trait loci (QTL). Recently there have been numerous progresses in whole genome sequencing, in the development of next generation sequencing technologies and high throughput genotyping platform. Advances in these technologies have led to the foundation of the high density single nucleotide polymorphism (HD-SNP) arrays as an up-to-the-minute implement for the genetic and genomic analysis of farm animals (Fan *et al.,* 2010). There is a rapid development and progression of fam animals genomics has introduced novel technologies skilled presenting global description of biological system at the level of gene and protein expression (Bendixen *et al.,* 2005). In the past few years, an innovative technology known as microarray has attracted fabulous interest among biomedical and biological researcher. This technique can be used for gene expression analysis, polymorphism detection, DNA sequencing and genotyping on a genomic scale (Lemieux *et al.,* 1998). These all methods help us to increase our knowledge about the genetic architecture of complex quantitative traits in farm animals and to estimate the distribution of the genetic variation across and within breeds and population.

The objective of this paper is to review some advance genomic biotechnology applications in animal breeding. Here I made emphasis in both the search and use of genomic information for selecting animals for the improvement of livestock concern with future.

The potential of genomic technologies: The genomics technologies in livestock present a major and foremost opportunity to address the responsibilities of agricultural production to humanity at large. Animal form a distinctive genomics resource as a result of their significant phenotypic diversity and of their population structure which make them particularly furnish for positional cloning. These are the assembly of techniques used in genetic screening to identify the precise area of interest in genome. The purpose of genomic technologies is the characterization and mapping of the locus that affected these traits of interest (Koopaei and Koshkoiyeh, 2011). The current advancement in characterizing the genomes of animals, including the identification of large number of single nucleotide polymorphism (SNP) make a major impact on the identification of genes and mutation underlying this phenotypic diversity including diseases susceptibility, morphology and behavior. With advance in single nucleotide polymorphism (SNP) and genotyping technology and combined with innovative statistical genetics procedures floor the approach towards genomic selection. The progress in technology has promoted the whole genome sequencing of organism, comprising mammalian model species important for human health, agriculture and flag species for wildlife conservation (Archibald *et al.,* 2010). Millions of genomic DNA variation included point mutations, deletions, insertions and segmental duplications discovered through whole genome sequencing and the following re-sequencing HapMap project (the bovine genome sequencing and analysis consortium *et al.,* 2009). There have been exposed that marker panels comprising many thousand to more than a validated SNPs have been developed from these effort (Ajmone-marsan, 2011).

Recently genomic and bioinformatics advances have also created reasonable opportunities to researchers to characterize livestock species in term of function of their genes (McCarthy *et al.,* 2009). Genome sequencing, expression array, single nucleotide polymorphism (SNP) maps with automated genotyping and database management are speedily becoming valuable constituents of our genomic toolbox. These technologies are used in providing assure for genome mining and gene discovery. DNA sequencing and SNP identification in domestic animals species will provide us information about linkage disequilibrium over large genomic regions and the identification of haplotypes blocks in various population and breeds of livestock. RNA interference (RNAi) is an innovative tool for experimental modification of gene expression in animals and may possibly soon locate its approach into animal improvement.

Livestock Genomics Architecture: Livestock have provided a high quality and passionately accessible protein source for human consumption since their domestication. In addition man has constantly modified the genomes of these species through a variety of selective breeding practices for trait ranging from growth, color, composition and disposition. These all selective breeding practice of animals was result from phenotypic information that have tremendous potential for unblocking the secrets and furtive hidden in the billions of bases that make up an organism's genome. Animal genomics studies are very concern with challenges, in relation to the increase worldwide demand for high quality and healthy food, the prerequisite of sustainable economics and environments of breeding system and want to adapt to global changes (Charles *et al.,* 2010). In order to face the challenges, it allows acquiring standard knowledge on the structure and functioning of domestic animals genome, obtained from molecular data using very efficient technologies. As an accompaniment to genomic data the achievement of phenotypic data as measure of various traits at different stages of their biological development, presents enormously well and defined characterization of the animals studied in different environment (Hume *et al.,* 2011). The combined use of these different types of both genomic and phenotypic data makes the complex biological interaction and regulation that control animal characteristics and variability.

Use of genomic information in selection: "the detection of selection signature": Knowledge about the

genome of livestock will be valuable to agriculture, relieves on improving the genotype of the animals and improving their management. The application of new-fangled genomic technologies is getting awfully significant impacts in the livestock industries globally. In farm animals using genomic selection increased the correctness of selection and having a great impact on shorten the generation interval and in the more comprehensively it rapidly replacing the traditional quantitative genetic approaches based on general progeny testing (Zhang, 2011). Genomic regions under selection having an applicable significance in conservation as well as in disentangling the genetic basis of complex traits (Ajmone-Marsan, 2011). A number of techniques have been urbanized for the detection of selection signature (Oleksyk *et al.,* 2010). The prior availability of SNP markers, genome wide selection signatures have been frequently explore in humans, using both sequence as well as genome wide marker date (Oleksyk *et al.,* 2010). The necessitation to precisely envisage the best performance of animals across multiple breeds, the genomic technologies based selection play role preeminent. The use of genomic information e.g. sequence or DNA marker polymorphism, for the selection of farm animals need the knowledge of the effect of physically mapped genes with effects on economically significant traits or quantitative trait loci (QTL) (Montaldo, 2006). Genome wide selection using a large number of SNP markers across the intact genome with phenotypes to build the accurate estimation of breeding values (EBVs) for candidates to selection (Meuwissen *et al.,* 2001). In addition, Genome based selection of farm animals have an immense prospective to diminish the inbreeding rates (Dekkers, 2007) and can appreciably help in quantitative trait genetic architecture (Daetwyler *et al.,* 2010).

Molecular Marker technologies: A tool for exploiting genetic diversity: Benefiting from the PCR techniques, the Molecular markers have now become a fashionable ways for the identification and characterization of animal species. Molecular marker technologies encompass protein based and DNA based techniques, the later is generally preferred as compared to the earlier protein based markers, because of showing low susceptibility to environmental or developmental influences. DNA markers correspond to a short sequence of DNA, the presence of which is linked to a desirable trait (Williams, 2005). In the last decades a number of marker techniques were consequently developed, in particular RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), RAPD (randomly amplified polymorphic DNA), microsatellite (simple sequence repeat), SNP (single nucleotide polymorphism) and mitochondrial DNA markers. The presence or absence of markers allows the genotyping of individuals and populations. Genetic markers can get a number of forms, polymorphic in nature and associated with a particular traits or genes (Williams, 2005). Molecular genetic markers could be used to estimates the genetic diversity within or between the breeds (Troy, 2001 and Hanotte, 2002), presents information of concern allelic variation at a given locus (Erhardt and Weimann, 2007). The use of genetic markers to illustrate the genetic make up and forecast the performance of an animal is a powerful aid in animal breeding (Beuzen *et al.,* 2000).

Restriction Fragment Length Polymorphisms (RFLP): The restriction Fragment Length Polymorphisms (RFLP) was the earliest form of deoxyribonucleic acid (DNA) marker used to build up the first true genomic map (Williams, 2005). This hybridization based marker technology use synthetic oligonucleotides as probes, which are fluorescently labeled to hybridize DNA (Teneva, 2009). RFLP technology and was first developed in 1980s (Botstein *et al*., 1980). Use the restriction enzymes that cut the DNA at specific site to visualize the differences at the level of DNA structure (Mburu and Hanotte, 2005). By using RFLP differences are marked when the length of DNA fragments are different, its mean that the RE (restriction enzymes) cut the DNA at distinct locations. The change or polymorphism occurs due to mutation imply creation or eliminating of the RE site and make new RE site. The variations are identifying by using a hybridization probe. Molecular biology lab apparatus gel electrophoresis is requires for the identification of RFLP to separate the DNA fragments of different length to transfer the fragments to nylon membrane and radioactive labeled probe is used to visualize the DNA fragments exposed to an X-ray film (Lien, 2001).

Microsatellite marker: Microsatellite are short sequences made up of a simple sequence motif, 2-6 bases long tandemly repeated and arranged head to tail without interruption by any other base or motif. Microsatellites loci are also known as short tandem repeats (*STR's*), simple sequence repeats (*SSR's*) and simple sequence tandem repeats (*SSTR*). They are interspersed throughout genome, the repeated unite are varies among individuals and they have adequately high mutation rate. The microsatellite precious as genetic marker being co dominant in nature and can also detect high level of genetic or allelic diversity (McCouch *et al.,* 1997).

Currently these are the most well-liked markers in livestock genetic characterization studies (Sunnucks, 2001). Their high mutation rate and co-dominant nature make them useful marker in the estimation of within and between breed genetic diversity. For microsatellite data analysis, there is some controversy has surrounded the choice of a mutation model, infinite allele or step wise mutation model (Goldstein *et al.,* 1995). However the imitation of studies has shown that the infinite allele

model is normally valid for assessment of within species diversity (Takezaki and Nei, 1996). Through microsatellite analysis there are different parameters included mean number of allele (MNA), observed and expected heterozygosity (Ho and He) are the most common for assessing within breed diversity. The second most significant factor assessing diversity is the multivariate analysis, the more recently the Bayesian clustering approaches have been proposed, generally used being FST (Weir and Basten, 1990), measure the degree of genetic differentiation of subpopulations through calculation of the standardized variance in allele frequencies among populations.

Microsatellites marker being of choice in livestock for many kinds of molecular applications including, genetic characterization studies (Civanova *et al.*, 2006), analysis of population structure (Arora *et al.*, 2004), to estimate genetic variability and inbreeding (Mateus *et al.*, 2004), evaluation of paternity (Luikart *et al.*, 1999), phylogenetic relationships among populations (Saitou and Nei, 1987), disease diagnostics, forensic analysis, development of genetic map, Marker assisted breeding (Montoya *et al*., 2007; Ritz *et al*., 2000 and Naicy and Anilkumar, 2008).

SNP (Single Nucleotide Polymorphism), "A valuable and efficient molecular marker": Recently there have been numerous progresses in whole genome sequencing, in the development of next generation sequencing technologies and high throughput genotyping platform. Advances in these technologies have led to the foundation of the high density single nucleotide polymorphism (HD-SNP) arrays as an up-to-the-minute implement for the genetic and genomic analysis of farm animals (Fan *et al.*, 2010). The SNP have been represent one of the more interesting advance in genotypization. They are abundant in the genome, genetically stable, found in both coding and non-coding regions, biallelic co-dominant marker and disposed to high-throughput automated analysis (Vignal *et al.*, 2002 and Stoneking, 2001). SNP present in coding regions can be frankly associated with the protein function and as the inheritance pattern is more stable, they are more suitable markers for selection over time (Beuzen, 2000). The importance of SNP in a restriction enzyme recognition site is also confirmed by PCR-RFLP, used as genotyping procedure and the PCR product as a result of restriction enzyme cutting will generates a varying fragments can be analyzed usually be gel electrophoresis. Due to the appropriate coverage and density over the genome, SNP could contributed to (1) it is a tool for more proficient genotyping, species identification and evaluation and for further analysis of population structure and genetics (2) exploring the genetic mechanism of complex agricultural traits (3) more amenable tool improving selection method for genetic improvement of farm animals production. In July 2010, Illumina released two new genotyping SNP chips including a low-density chip (Bovine3K) having 2,900 SNP (Illumina, 2010c) and a high density chip (BovineHD) with 777,962 SNP (Illumina, 2010a) and in January 2011 Affymetrix released a high density chip with 648,855 SNP (Wiggans, 2011). Although such chips can provide genotypes that enhance the precision of genomic evaluation by better tracking of the loci responsible for genetic difference (VanRaden and Tooker, 2010). SNP chips are currently available for human, ovine, bovine, canine, porcine and equine species (Ajmone-Marsan, 2011).

Genotyping technologies: In the last decade there have been massive advances in genotyping technology, but due to technological limitation, approximately all genotyping is partial, through these technologies only a small fraction of an individual genotype is determined rather than the whole genome' genotype. In the near future the new innovative e.g. Illumina's Human-1 BeadChip or mass sequencing technologies have promise the whole genome's genotyping. Multicolor fluorescence detection ability of CE (capillary electrophoresis) instrument such as ABI PRISM genetic analyzer have a leading role in STR genotyping, the effort is going to develop microchip platform (Liu, 2007) to perform high resolution DNA genotyping. In addition mass spectrometries (MS) with matrix assisted laser desorption/ionization (MALDI) and elctrospary ionization (ESI) techniques have been used for STR typing without allelic ladder (Butler, 1998). There are a number of genotyping techniques available, capillary electrophoresis based genotyping techniques includes AFLP®, ISSR (inter simple sequence repeat) analysis, Relative fluorescence quantification, Resequencing heterozygote detection, Single Sequence Conformation Polymorphisms (SSCP), Polymerase chain reaction, Confronting two pair primers (PCR-CTPP), Melting curve analysis of SNPs (McSNP®) and copy number analysis (CNA) have been play their role.

Sequencing technologies -the Next generation: Always demand has been greater for technologies that have ability to quickly, accurately and inexpensively sequence the genome of an individual and provide accurate genomic information. These challenges have been catalyzed the development of next generation sequencing (NGS) technologies. It opened fascinating opportunity in the life sciences, providing a detailed analysis of individual genome stretches accurate analysis of RNA transcripts for gene expression and offer novel and quick ways for genome wide characterization, transcription factor regions, and structure of chromatin, DNA methylation patterns and metagenomics (Ansorge, 2009). The NGS approach also identifies if the alternative exons of a gene are used to generate diverse forms of a protein in different tissue and also present the DNA sequence of each transcript (Rolf, 2010). Both RNA-seq and DNA

sequencing data provide impending towards novel and casual polymorphism within an individual. NGS platforms distributed a common technological aspect massively parallel sequencing of clonally amplified or single DNA molecules that are spatially separated in a flow cell. This design is a archetype shift from that of Sanger sequencing, which is based on the electrophoretic separation. As a especially parallel process, NGS sequencing hundred of megabases to gigabases of nucleotide sequence output in a single instrument run, depend on the platforms, included ROCHE/454 life sciences, Illumina genomic analyzer, Applied biosciences/SOLiD and HELICOS biosciences and single molecule sequencing.

Sequencing have to extend our knowledge on SNP variation, find out rare alleles and alleles detained in particular breeds and the other polymorphisms such as insertions, deletions and copy number variation (CNVs) have the potential to affect animal phenotype (Beckman *et al*., 2007).

Application of next sequence generation technology (NGS)

1) Identification of actual expression level of all the genes that are expressed in essentially any tissue and provides quantitative data to identify differences in gene expression between two samples
2) To detect functional genes and markers of important traits to facilitate molecular breeding and improve agriculture production and conservation.
3) NGS offer novel and quick ways for genome wide characterization, profiling of RNAs, transcription factor regions, DNA methylation patterns and metagenomics.

Integration of MAS (Marker assisted selection) in breeding programs: The fast development of molecular techniques has opened up foundation of genes to animal breeding that were not available before through conventional breeding, creating a lot of interest about MAS (Marker assisted selection). The advance use of molecular genetic technologies prospectively presents the way to select the breeding animals at an early stage (even embryo); to select for a superior variety of traits (Naqvi, 2007). Animal researchers are currently formulating their trust on genetic markers. These markers have no function of their own; they are sections of the genome of an organism in question which are used for recognition. They can simply understood as naturally occurring tags unite to a particular region of the chromosome, this region will enclose hundreds of genes, but we do not generally know which genes they are of what their function is (Foolad and Sharma, 2005). To discovered the effect of the genes on the phenotype of animals, we can follow the inheritance of these markers in families of animals and see whether inheritance any of these is associate with the develop performance. If they are, then we presume that one or more genes in this region of marker are associated with beneficial effects. Then we use the information on the genetic markers to make future selection decisions, so the any animal that inherits the marker will also inherit the valuable effect correlated with it (Naqvi, 2007), this is known as marker assisted selection (MAS). In genome wide association studies (GWAS), both the candidate gene and QTL (quantitative trait loci) mapping strategies have been widely utilized in domestic animals for the finding of genetic markers appropriate for MAS (Fan *et al.,* 2010).

At the forefront of quantitative genetics: Quantitative genetics has been used for many years in selection of animals for higher productions e.g. yield growth and efficiency. Quantitative genetics is concerned with the variability in complex traits that is caused by the correlated outcome of variant alleles at several loci and as well as non genetic factors or environmental factors (Charlesworth, 2005). Most of the traits of animal genetic improvement programs are quantitative traits, those under polygenic control (control by many genes) and frequently display continuous phenotypic variation within or among population (Hu, 2009). Because of the measurability and high heritability, livestock genomics will have a considerable impact on developments in quantitative genetics. There are numerous corresponding approaches to identify markers for these multifactorial traits, including linkage analysis, association analysis and genome wide scans. Genes that influence quantitative traits are known as quantitative trait loci (QTL).

QTL- mapping: There is a massive advance in molecular marker analysis, so it is now significantly promising to analyze both the simply inherited and quantitative traits and identify individual genes controlling the traits of concern. The exploitation of genomic information in livestock improvement involved the location of all markers and protein coding genes in the chromosome (Montaldo, and Meza-Herrera, 1998). Therefore the development of genetic maps of the species is required for detecting QTL using genetic markers (Bovenhuis *et al.,* 1997). Molecular markers could be used to mark quantitative trait loci (QTL) to appraise their contribution to the phenotype by selecting favorable alleles at these loci in a marker assisted selection scheme aiming to accelerate the selection and genetic advances (Angaji, 2009). QTL loci play a significant role in farm animals, as they can further the credentials of traits related to milk and meat production. The significant objectives of the genome research in farm animals is to map and characterized genes that are causative for QTL. Basically there are two QTL mapping strategies, association test using candidate gene and genomic scans based on linkage mapping in a cross population (Andersson, 2001). These two approaches have been used to identify the genes affecting trait of interest.

Candidate gene approach: The candidate gene approach studies the relationship between the trait of interest and known genes that may be contained in the physiological pathways underlying the trait (Lui *et al.,* 2008). Candidate genes are the genes with known biological function directly or indirectly regulating the development processes of the production traits, which could be confirmed by evaluating the effects of the causative gene variants in an association studies (Zhu and Zhao, 2007). It was suggested as a process to identify genes with imperative phenotypic performance effects for possible use in the improvement of farm animal productivity (Streelman and Kocher, 2000). Due to numerous advances in biotechnology which increased the extent of genomic information significantly facilitates candidate gene analysis in domestic animals. Candidate genes can be elected based on both their biological role as well as their genomic position (Schulman *et al*., 2009). Recently the most outstanding progress in this field is the manifestation of digital candidate gene approach (DigiCGA). This approach also named in *silico* candidate gene approach or computer facilitated candidate gene approach, is a novel web resources based candidate gene identification approach. The completion and development of the animal genome projects have exposed a huge number of potential opportunities for identifying candidate genes in which digital approach is an attention-getting one and as such could enable the systematic identification of genes underlying biological traits (Glazier *et al.,* 2002). The DigiCGA can be described as an approach that analytically extract, filter, (re)assemble, or (re)analyze all possible resources available derived from the public web database mainly in accordance with the principle of biological ontology (BO) and complex statistical method to make computational identification of the candidate genes of specific interest, which is generally followed a subsequent validation of actual association analysis (Zhu and Zhao, 2007). Currently some application software or online tools for prioritizing candidate genes such as GFSST (http://gfsst.nci.nih.gov), ENDEARVOUR (http://www.esat.kuleuven.be/ endeavour), POCUS (http://hgu. mrc.ac.uk/ users/ Colin. semple/), G2D (http:// www. ogic.ca /projects/g2d-2/), QTL Mixer (http://qtl.pzr.uni-rostock.de/qtlmix.php) (Zhang *et al.,* 2006; Aerts *et al.,* 2006; Turner *et al.,* 2003 and Perez *et al.,* 2005) and others have been developed and released to public.

Genome scan approach: The genome scan approach studies the relationship between a trait and markers selected across the genome to identify chromosomal locations associated with the trait (Andersson, 2001). This approach will find the map location of a trait locus with a vital effect, it involved the following steps (1) design and construction of resource population, (2) phenotyping traits of resource population (3) selection of genetic markers (4) genotyping of the population for selected markers (5) construction of linkage maps and (6) statistical analysis of the phenotypic and genotypic data derived from the resource population (Da, 2003 and Kadarmideen *et al.,* 2006). Using the genome scan approach a huge amount of QTL can be achieved in farm animals that can provide a useful association to link genomic information with phenotype.

Reproductive technology in animal breeding: Biotechnology has been directed primarily towards reproductive technology have been employed for genetic improvement of farm animals which is foremost concern over the years for scientist and researchers. Advances in assisted reproductive technologies (ART) like Artificial insemination, In vitro Production, Superovolution, Embryo transfer, transgenesis and cloning have become significant in livestock breeding, have been introduced to overcome reproductive problems (Vikrama and Balaji, 2002). All these technologies able to speed up genetic changes due to shorter generation interval and improving accuracy in selection program (Anonymous, 1992). The aim of reproductive technologies in animal breeding is overcome the ambiguity about the true genetic merit of breeding animals. ART is a general term which is used to achieve pregnancy by artificial means. It's aimed and application in routinely used today of reproductive technology in the treatment of infertility.

Artificial Insemination (AI) and embryo transfer (ET) are probably the most well known methods that have been adopted in developed and developing livestock production (Kahi1 and Rewe 2008). The recent advances in biotechnology technologies in reproduction included production of transgenic animals and cloning (Smidt and Niemann, 1999). RT has prolonged effects on animal breeding in the future, as the increases the rate of reproduction and decrease the generation time (Abu *et al.,* 2008). The most successful reproductive technologies like AI and ET necessitated applying on large extent, some emerging biotechnologies such as Multiple Ovulation and Embryo Transfer (MOET), In Vitro Fertilization (IVF) and cloning provides prevailing tool for rapidly changing the animal populations genetically. These technologies will absolutely play an imperative role in the future perspective and visions for efficient reproductive performance in livestock (Vikrama and Balaji, 2002).

Transgenic technology: Transgenic animal technology is in the practice of revolutionizing the manner we domesticate the livestock. The transgenesis means transfer of foreign gene (gene of interest) into the genome of other species in a way that it stably passed from generation to generation. It is been a potential way in accelerating and facilitating genetic improvement in livestock. The process to produced transgenic animals initiated with the purpose of producing better breed lines,

which are strong, more caracass, high growth rate and increase milk production (Venkatesh, 2008). In breeding, transgenic animals are created to improve qualitative and quantitative treats in livestock and to reduce susceptibility to diseases (Duszewska1 *et al.,* 2010).

This technology uses the transgene encoding a particular trait is clone into a vector which may be synthetic, virus or plasmid DNA, and hybrid vector is inserted into the genome of the host organism. A variety of methods have been developed to produce transgenic animals, some have had much success and others are being further researched. There are several methods have been introduced to create transgenic animals, in these the most common method is the microinjection of a transgene into pronucleus of a newly fertilized egg, the introduction of desired gene into embryonic stem cells and the transgenic somatic cell nuclear transfer (TSCNT) which is the variant of SCNT.

Pronuclear microinjection: The microinjection into pronucleous is the most common method known in microinjection of exogenous DNA into the pronucleus of a newly fertilized egg (zygote). This technique is used to produce transgenic sheep and pigs (Hammer *et al.,* 1985) and also transgenic cattle (Krimpenfort *et al*. 1991). Using this technique earliest successful creation of transgenic mice was reporated in 1980s (Gordon *et al.,* 1980). In this technique of transgenesis, animal sperm and egg are united by *in vitro* fertilization (IVF) (Bailey, 2010). The newly fertilized zygote before dividing has one male and one female pronucleus. Both male as well female nuclei is use for microinjection, but male nuclei which is larger is often preferred. A glass micropipette pulled have a very small diameter is used, with micropipette cell membrane is penetrated without causing any damage. In this technique many copies of genes are inserted into the donor nucleus, there is no control of the incorporation of transgenes into the genome of the host. The successful microinjected zygote is transfer into the uterus of a psuedopregnant foster mother. Because all cells of any organism is derive from the zygote, if this technique gets a positive result then the transgene will be present in all cells, thus it will create a transgenic lines, so these are identified as germ-line transgenic animals (Harper, 1999).

Sperm Mediated Gene Transfer (SMGT): Sperm mediated gene transfer (SMGT) is an alternative technique using natural ability of spermatozoa as a vector to transfer exogenous DNA into the egg at fertilization (Bacci, 2007; Lavitrano *et al*., 2002 and Zani, *et al.,* 1995). This technique was first introduced in mice by Dr. Lavitrano in 1989. In this technique the sperm cells are stripped with DNA of interest, which binds to the surface of the sperm through specific protein-protein interaction. The DNA correlated with the sperm is then incorporated via protein dealings into the sperm nuclei (Zani, 1995). The sperm then act as a vector carries the genetic materials into the oocyte to incorporate the foreign DNA. There are several methods have been attempts at delivery of foreign DNA to the head of the sperm, including electroporation, liposome and plasmid delivery (Celebi, 2003). Recently one of exciting breakthrough in delivering of foreign DNA into the head of the sperm using monoclonal antibody mAb C. The positively charged basic linker protein monoclonal antibody is bind to negatively charged DNA through ionic interaction, which specifically binds the foreign DNA to sperm. The linker based sperm mediated gene transfer method (LB-SMGT) if improve is one of the best way to created the transgenic animals (Chang *et al.,* 2002).

DNA recombination in embryonic stem cells: Embryonic stem cells (ESC) have achieved major consideration in recent years in the field of medicine, agriculture and biomedical research due to their unique property of pluripotency. ES cells are derived from inner cell masses (ICM) of embryo at blastocysts stage. This type of embryo manipulation is used when inserting a transgene into a specific location in the genome (Bradely and Brosius, 2006). Two complementary strategies have been considered for the insertion of transgene in ES cell: homologous recombination and integrase mechanisms (Norman and MacInnes, 2002). With the introduction of homologous recombination the scientists and researchers are able to restore gene fuction (knock-in animals), take out gene function (knock-out animals), inactivated, or introduce any alteration in gene of interest (Mullins and Mullins, 1996). In vitro the gene of interest is inserted into ES cells by microinjection, viruses, electroporation or chemicals (http://users.rcn.com/jkimball. ma.ultranet/ BiologyPages/ Transgenic Animals. html). Once the intended genetic change has been screened, the suitable Es cells are injected into blastocyst of developing embryo, which is implanted into the uterus of surrogate host (Mullins and Mullins 1996). At this stage it is possible to injecting cells into blastocyst to obtain chimeras (Kakar, 2004). Chimera's production is an interesting area of biotechnology. It is an organism having more than one genetically different population of cells that originated from more than one zygote. In modern chimeric animals, culture cells derived from one strain of organism are injected into embryos of another strain of organism by direct embryo aggregation or by introducing into the blastocyst stage of embryo. The resulting organism has tissue derived from cells of both strains (www.criver.com).

Cloning Technology: Cloning is a significantly useful breeding tool, considering a perfect way to improve the performance of farm animals. One of the principle purposes of cloning is to increase the number of species in a population with superior characteristics. Cloning technology has concerned the interest of breeders for

many years. Animal cloning is the most topical development of selective assisted breeding in livestock (Wells, 2003). Cloning has been used to replicate elite breeding animals (Plume, 2009). As Dolly the sheep was the first animal to be cloned in 1996 (Wilmut *et al*,. 1997) by Somatic cell nuclear transfer (SCNT). Since that time many other species have been cloned by the same process. According to Plume K. (2009) there are around 6000 farm animals' clones worldwide. The cloning technology has been applied in the breeding of elite cattle (Kato *et al.,* 1998), goat (Baguisi *et al.,* 1999), pig (Polejaeva *et al.,* 2000), horse (Galli *et al.,* 2003), buffalo (Shi *et al.,* 2007), camel (Wani *et al.,* 2010), Rabbet (Chesne *et al.,* 2002) and other pet species like dog, cat, rat, ferret, mouse (Wakayama *et al.,* 1999; Roslin Institute online, 2003; Lee *et al.,* 2005; Li *et al.,* 2006 and Shin *et al.,* 2002). Cloning is an asexual reproduction of genetically identical organism can be achieved by nuclear transfer (NT) or by embryo splitting (Abu *et al.,* 2008). High cost of cloning is the factor that limited the technique uses in practical animals breeding (Hugo, 2006). Cloning as compared to other assisted reproductive technology its effectiveness remains significantly little, there are notable species difference make the consequence of this technology significantly different.

DNA Microarray technology: Assisting in breeding decision: A DNA microarray is a complex technology used in molecular biology and medicine. A variety of techniques have been available to identify difference in gene expression exist, including subtractive hybridization, differential display, serial analysis of gene expression and microarray hybridization. Some of these methods have been used to explore changes in gene expression in livestock (Moody 2001). Microarray technology has become one of the significant tools, scientists and researchers used to monitor genome wide expression levels of genes in a given organism. It was initially developed as a consequence of the increasing need for large scale DNA mapping and sequencing to facilitate the study of complex cellular process (Hoheisel, 2006). DNA microarray also known as DNA/RNA Chips, BioChips or GeneChips are a collection of DNA segments, immobilized on a solid surface (e.g. glass, plastic or silicon chip) which allow the quantitative and high throughput analysis of several genes through hybridization to a set of specific probes. Each particular hybridization reaction on an array is referred to as a spot or feature, and a characteristic array may include thousands of spots (Walsh and Henderson 2004).

Microarray is novel technologies have attracted a huge deal of attention from animal geneticists and breeders. It has been used for large scale gene expression studies and the high throughput system makes genotyping efficient and low cost, particularly for single nucleotide polymorphism (SNP) and indel polymorphisms (Galbraith, 2006). DNA microarrays are widely applied in genome wide genotyping for identifying variation (Petricoin *et al.,* 2002). Whole genome genotyping tools based on SNP markers are now available as microarray based genotyping arrays (*from www.affymetrix.com and www.perlegen.com*). These arrays are now allocated genotyping for the entire genome with tens of thousands of SNP markers in a single hybridization step, so it appreciably increasing the throughput and decreasing the cost of current gel based techniques for molecular mapping (Kadarmideen, 2006). These expression studies have been carried out to compare gene expression of breeds (McCarthy *et al.,* 2009). Relatively apart from gene expression profiling to established fingerprint of an animal with desirable characteristics, direct genotyping of DNA for variants associate with genes that produce desirable traits will be of interest (Feilotter, 2004). The DNA microarray used for this purpose would be allow the large scale screening of many hundred of such markers in a sole experiment, allowing selection based on multiple traits. The newly genomic approaches such as DNA microarray, SNP discovery and genotyping are hopeful tools for improving and advancing farm animal breeding.

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