The Role of Existing and Emerging Biotechnologies for Livestock Production: toward holism

Matthew B. Wheeler 1,2,3, Elisa Monaco1,2, Massimo Bionaz1,2 & Tetsuya Tanaka1,2

ABSTRACT

Animal biotechnology has been practiced in one form or another since the beginning of the domestication of animals. Many of the previously used tools of animal breeding, genetics and nutrition have played an important role in the selection, propagation and management of desirable and economically important characteristics in livestock. Modern livestock production has been dependent on biotechnology for development of improved feedstuffs, feed ingredients, vaccines, biologicals, enzymes, high quality genetics, genetic markers and assisted reproduction.

Recently, new technologies including genomics, transcriptomics, proteomics, metabolomics have been applied to livestock production. The term “omics” refers to a broad field of study in biology and stems from “Omes”, the Greek for ‘all’ or ‘complete’. Therefore, these technologies offer a holistic instead of a reductionist view of the biological phenomena. Several “omics” technologies are readily available for scientists or industry today and in this section we will provide a brief overview of their availability in livestock science. Other “omics” technologies have developed quickly and are available for research or industry in livestock field. The microarray technology for microRNA is available today for bovine, pigs, and chickens. Combined with the use of appropriate bioinformatics tools, they have been of great help in understanding livestock genomics. Large-scale SNP arrays are also available today, but only for bovine among the livestock species. Epigenomics, the study of the non-DNA hereditable factors affecting the phenotype, has been used for large-scale studies, but data have not been generated using this technology in livestock. Systems biology has emerged to investigate “interrelationships of all of the elements in a functioning system in order to understand how the system works”. A systems biology approach is only possible by combining a single or multiple “omics” technique(s) along with bioinformatics for a broad purpose such as to study the whole system, organism or comparison between organisms.

In order to take full advantage of the breakthroughs from “omics” techniques efficient animal breeding and reproduction of these rare genetic individuals needs to take place. For decades assisted reproductive technologies (ART), such as artificial insemination (AI), superovulation (SOV), embryo transfer (ET), and in vitro embryo production (IVEP), have contributed to animal breeding programs allowing faster transmission of desirable traits in livestock populations in a shorter period of time compared to classical approaches. The use of transgenic technologies along with ART’s to introduce single or multiple genes into existing genomes of livestock has played an increasingly larger role in the genetic development of our production livestock. Addition of appropriate stem cell technologies to the genetic “toolbox” has further increased our capabilities to enhance and modify livestock genomes and physiology.

In the future, livestock production will rely even more heavily on existing and emerging biotechnological advances to produce our food. However, improvements are still needed in product composition and production efficiency, especially in growth, disease resistance, and reproduction. The attainment of such improvements will depend heavily on our ability to quantify desirable traits, to identify markers linked to gene(s) responsible for those traits, to select or redesign populations of superior individuals, and to propagate those animals efficiently, practically and economically.

Keywords: Genomics, proteomics, systems biology, embryo transfer, transgenics, stem cells

1Department of Animal Sciences; 2Institute for Genomic Biology; 3Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana IL 61801, U.S.A. Department of Animal Sciences, University of Illinois, 1207 West Gregory Drive - Urbana, IL 61801, USA - CORRESPONDENCE: M.B. Wheeler [E-Mail: mbwheelee@illinois.edu or mbwheelee@me.com – Tel: (217) 333-2239].

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I. INTRODUCTION

This review will examine the role of “omics”, assisted reproduction, genetic modification of livestock, stem cells and future technologies that may be applied to livestock production. The selected topics were chosen to give an overview and future potential of these methodologies for incorporation into our livestock production schemes. Many of these tools will be refined, expanded and improved. Further, it is anticipated that new tools and technologies will be developed and deployed to the field.

II. “OMICS” IN LIVESTOCK PRODUCTION AND REPRODUCTION

The term “omics” refers to a broad field of study in biology such as genomics, proteomics, or metabolomics. “Omes” stems from the Greek for ‘all’ or ‘complete’. Therefore it offers a holistic instead of a reductionist view of the biological phenomena. Several “omics” technologies are readily available for scientists or industry today and in this section we will provide a brief overview of their availability in livestock science. The development of “omics” technologies was a consequence of progress in cellular biology. Each “omic” technology is a means to quantify the major factors at play in the cells (Figure 1).

Genomics

The term genomics strictly considers only the study of the genome with major emphasis on DNA sequencing [65]. The initial sequencing of genomes was extremely expensive and the technique used, the automated Sanger sequencing, was burdensome with many steps, and these steps were technically difficult to improve [96]. Today, new technologies have been developed for DNA sequencing, called next-generation sequencing, which can provide a fast, accurate, and relatively inexpensive sequencing of entire “personalized” genomes [96-97]. Those new technologies are already changing the way we look at the genome and will allow, among others things, measurement of the whole transcriptome, the non-coding RNA, and genome-wide single nucleotide mutations (or polymorphisms) [97]. In addition, the next generation sequencing can be coupled with other technologies (e.g., CHIP ChiP, see below) to study gene regulation by transcription factors, DNA methylation, histone modification, and DNA accessibility/chromatin structure [97]. From a livestock point of view, the use of this technology will dramatically increase the speed and accuracy of genome studies, both as functional genomics and molecular-based selection for desirable traits. Species for which genome sequence is not available will particularly benefit from these technologies.

Transcriptomics

The measurement of the transcribed genome (transcriptomics), particularly for the protein-coding, transfer, and ribosomal RNA has experienced tremendous technological progress in the last decades. The simultaneous measurement of thousands of transcripts, if not the whole transcriptome, is possible today by microarray platform technologies [11]. The progress of those technologies was a consequence of the whole genome sequence projects. Application of large transcriptomic analysis in livestock is still relatively scarce, as partly summarized by Hocquette et al. [65], but will likely increase in the next few years. The completion of genome sequences in livestock species will
Figure 1. This figure is a schematic representation of the major “omics” technologies (left) currently available for quantification of cellular phenomena (right). The use of such technologies together with bioinformatics allows for a systems biology approach to study cells, tissue and systems. These combined technologies should provide a real-time picture of the cell’s biology and should allow capture of all the possible effect(s) of a physiological state, drug exposure, nutrient, physical interaction and other chemicals on the cell/tissue. The systems biology approach does not only consider the quantification of the various phenomena, but allows for the study of all the interactions among them (i.e., networks and communications). Such analyses will ultimately reveal living processes at the cellular level.

almost certainly allow for measurement of the whole transcriptome. Whole transcriptome sequencing using the next generation sequencing technologies is rapidly developing, which will provide another means to measure the abundance of all transcribed RNA including non-coding RNA and splice variants [45]. The advent of the next-generation sequencing appears a more appealing technology for the whole transcriptome analysis compared to the microarray technology [142].

Exploiting genomics for livestock selection

The identification of DNA loci through specific DNA markers, which contain the information for quantitative traits, termed quantitative trait loci (QTL), has improved the efficiency and efficacy of selection [153]. QTL however, are large regions of DNA containing several protein-coding genes and only a few genes have been successfully identified and demonstrated as being responsible for specific quantitative traits (reviewed in [117]). The advent of large scale analysis of single nucleotide polymorphisms (SNP) has increased the potential of identifying specific genes responsible for the trait in the QTL, passing from QTL to QTN (quantitative trait nucleotide(s)). The same technique also allows for evaluation of the potential role of the non-transcribed DNA [117]. Now that the bovine genome has been fully sequenced and large numbers of SNP are known, the genome-wide scan to identify quantitative trait loci for several traits has been performed [48]. Some examples are SNP related to milk fat composition [120,134] and milk protein composition and protein percentage in cattle [121].

Further, the demonstration of the accuracy of selection solely based on analysis of dense SNP DNA markers was the basis for the birth of the genomic selection. This genomic selection technique has already been demonstrated to substantially improve the selection in dairy cows (reviewed in [60]). The use of next-generation
sequencing will probably tremendously increase the discovery of QTN in livestock [128], as has been shown in rodents [142,151]. This will both accelerate and enhance selection for desirable traits.

The combination of large output, if not the whole genome, transcriptome analysis with the QTL (expressed QTL or e QTL) appears a promising tool to describe the variation affecting transcription abundance [68,126]. This method has been applied in yeast, rodents and humans, but not yet to livestock [68,126]. The QTL or QTN, even when the association has been fully demonstrated, only explain part of the genetic variance, probably because many of the quantitative traits are complex [48]. It is becoming more evident that several QTL are dependent on epigenetic status which is hereditable [30]. The integration of the genetic and epigenetic approach appears to be necessary in order to further improve selection [68].

Proteomics and metabolomics

The direct measure of the quantity and structure of proteins composing cell(s)/tissue(s) (i.e., proteome) and the quantity and type of metabolite(s) (i.e., metabolome) are considered more direct functional measurements of a biological system compare to transcriptomics. Techniques to measure large scale proteomics and metabolomics are developing quickly but are not yet widely used in biology, especially in livestock science.

The improvement of proteomics is crucial and several techniques are currently available to provide absolute and relative protein quantification (reviewed in [40]). Application of large scale proteomics in livestock has been useful for studying proteins composing milk fat globules and bacteria present in milk (reviewed in [46]), milk proteome after mastitis [14], and muscle quality [107]. The study of proteomics in livestock will probably benefit by the development of protein-specific antibody arrays [18,53] or ImmunoCell-Arrays [47]. However, the advancement of these technologies is challenging because of the limited availability of specific antibodies in livestock species.

Two metabolomics approaches exist: metabolic profiling and metabolic fingerprinting (reviewed in [65]). The metabolomics in the “omics” sense has been rarely used to date in livestock. To date, metabolomics has been used only to find anabolic hormones in bovine urine [116], to evaluate the effect of L-arginine supplementation in pig [61], and to investigate the effect of high-fiber rye buns in hypercholesterolemic pigs used for medical research [10]. Metabolic profiling in blood has been widely used in livestock [62,70]. Even though metabolic profiling in blood is not considered an “omics” technology, it has the potential to provide an overview of the metabolic, hormonal, and immunological status of the whole organism, therefore allowing a holistic view of the organism’s metabolism.

New “omics”

Other “omics” technologies are quickly developing and already available for research or industry in livestock field. The microarray technology for microRNA is available today for bovine [34], pigs [24], and chickens [6]. Combined with the use of appropriate bioinformatics tools [19], it can be of great help in understanding livestock genomics. Large scale SNP arrays are also available today, but only for bovine among the livestock species [25]. Epigenomics, the study of the non-DNA hereditable factors affecting the phenotype [16], is currently available for large scale studies [42,50], but to our knowledge, data have not been generated using this technology in livestock. Relatively new “omics” technologies are the phosphoproteomics [51], not currently applied to livestock and glycomics [105], which has been applied in studying the bovine milk glycome [138]. Glycomics has recently seen rapid technological improvement (e.g., the glycan array [85]). Other “omics” include the ChiP-Chip (chromatin immunoprecipitation technique associated with DNA microarray or DNA sequencing), which enables the study of genes regulated by transcription factors in “omics” scale [97], but has yet to be applied in livestock.

Nutrigenomics

Nutrigenomics can be defined as the holistic effect of nutrients on the organism/organ/tissue/cell using all “omics” technologies available. However, today the term mostly refers to the overview of the nutrient-gene interaction [65] and the effects on the network of genes/proteins (interactomics [22]).

The study of animal nutrition has been prolific in the last half century, particularly in dairy cows [32]. Most of the research in this field has been devoted to practical applications (i.e., modeling or systems to provide optimal dietary provision). While a lot of functional data have been generated by those studies, only a modest volume of molecular data has been produced. As previously proposed [32] the use of large “omics” in the study of nutrition will greatly improve efficiency and efficacy of using resources in animal production. Relatively few examples exist in
literature using “omics” to study nutrition in livestock [26,43,65]. Most of the studies have used transcriptomics, and very few have used bioinformatics approaches to provide functional interpretation of the data. Recent nutrigenomics studies were performed mostly in bovine [17,71-72,75,83,89,99,139], and the authors were able to find only one published study in chickens [27]. Nutritional science should take full advantage of the potential of using the nutrigenomics approach in order to understand the fine regulatory effects of nutrients.

Toward a systems biology approach

Systems biology has been considered to be the “5th great idea in biology” [145]. Systems biology (Figure 1) is defined as the investigation of “interrelationships of all of the elements in a functioning system in order to understand how the system works” [88,95]. A systems biology approach is only possible by combining a single or multiple “omics” technique(s) along with bioinformatics for a broad purpose such as to study the whole system, organism or comparison between organisms [86]. Systems biology considers not only the overall functional output of the parameters measured, but also tries to understand the interaction of the parts involved (i.e., interactome or networking). The study of the interactome is based on informatics managing and organization of scientific literature available through manually-curated datasets or automatic organization of information with a semantic-recognition algorithm approaches. The network of genes/proteins involved is essential to understand a system. Today, even though several tools have been developed in order to capture such information (e.g., Ingenuity Pathway Analysis™, Metacore™), the actual gain of knowledge from such analysis has been limited, particularly in livestock science. Even though limited, when such an approach was undertaken new information has been generated with practical applications in livestock, such as the discovery of the central role of PPAR\(\gamma\) in orchestrating milk fat synthesis [12,74]. Aside from the previous example, scientists using high-throughput methods in farm animals have not exploited the use of network analysis. At the root of this limited utilization of system biology in livestock science is the lack of understanding of basic bioinformatics by most livestock biologists. This lack of understanding prevents the full utilization of the available tools to interpret the data. There is a great need for bioinformaticists able to produce easy-to-use but also highly effective tools, as well as the need of a better bioinformatics preparation for scientists using large-output tools.

III. ASSISTED REPRODUCTIVE TECHNOLOGY

In order to take full advantage of the breakthroughs from “omics” techniques efficient animal breeding and reproduction of these rare genetic individuals needs to take place. For decades assisted reproductive technologies (ART), such as artificial insemination (AI), superovulation (SOV), embryo transfer (ET), and in vitro embryo production (IVEP), have contributed to animal breeding programs allowing faster transmission of desirable traits in livestock populations in a shorter period of time compared to classical approaches (Figure 2). However, the overall efficiency of these reproductive technologies remains somewhat low for a variety of reasons. In this section we will provide an overview of each of the abovementioned ART including underlying advantages, problems and future directions.

Production livestock reproductive technologies

Artificial insemination is the oldest and most powerful among the reproductive technologies because it is easy to perform, cost-effective, and highly successful [147]. For over 200 years AI has been used to obtain offspring from genetically superior males. Moreover, by the 1960s, significant improvements in cryopreservation and storage of semen made AI even more accessible to livestock producers [147]. In the modern dairy industry, where a large number of dairy cows are managed intensely, AI is widely used. Semen from bulls is especially amenable to freezing and long-term storage. In contrast, for reasons not yet well understood, semen from other livestock species such as horses, pigs, and poultry are more difficult to freeze and store.

Embryo transfer is a technique that allows obtaining more than one offspring per year from a genetically superior female. This technique allows producers to place increased genetic selection pressure on the female and benefit from superior female genetics much like that achieved through AI, although to a much lesser degree as many more offspring can be produced via AI from a genetically elite male. Depending on the species, embryos can be recovered from donor females of superior genetic merit by surgical or non-surgical techniques (the latter is only possible in cattle and horses). The recovered embryos are then transferred to recipient females of lesser genetic merit. To increase the number of embryos that can be obtained during an estrous cycle, the donor female can be treated with hormones to induce SOV. Moreover, to overcome the limitation of the immediate availability of suitable
recipient animals, embryos are often cryopreserved. However, pregnancy rates are significantly lower after cryopreservation than after transfer of fresh embryos [59]. There are many issues that still need to be addressed to improve the success rates of SOV and ET. One major problem is the variability among animals in responding to ovarian superstimulation and to the support of development of the transferred embryos. Beside genetic variation, nutritional status and fertility also play a role. One important aspect is the synchrony of developmental stage of the transferred embryos and the physiology of the recipients [100]. For example, this synchrony is important for success when oocytes are retrieved by ovum pick up from a donor female and matured and fertilized in vitro before being transferred into recipients.

In vitro production of embryos is feasible in many animal species with varying rate of success. The entire process (Figure 3) includes in vitro maturation and fertilization of the oocytes and in vitro culture of the embryos. The
oocytes can be collected by ovarian aspiration of slaughtered animals or by the use of ovum pick up techniques in live animals (cattle and horses). In vitro produced embryos can play an important role in genetic selection schemes because they can be screened for desired genes before pregnancy is established. In addition they can be used to improve pregnancy rates in low fertility animals [56]. However, several problems associated with IVEP have limited the appeal to livestock producers, such as sub-optimal quality of the embryos, low rate of fetal survival and occasional abnormal offspring.

Figure 3. Schematic diagram of the traditional microdrop in vitro embryo production (IVEP) system illustrating the different washes and medium changes that can occur.

Cryopreservation

With the development of ART the necessity of developing successful cryopreservation methods for reproductive cells and embryos became quickly evident. Semen cryopreservation has been and continues to be essential for international exchange of germplasm of genetically superior animals. Frozen semen can be used during AI and during IVEP schemes. In the 1950s, with the use of glycerol as cryoprotective, frozen bull semen methods allowed a great increase in the use of AI in the dairy industry [109,158]. Since then, there have been many attempts to implement various cryopreservation methods. The scientific literature has diverse examples of experiments on semen desiccation, vitrification and freeze-drying [73,81]. However, only limited improvement has been made [152] and the protocols currently used to cryopreserve sperm still provide suboptimal results. Bull semen has the best cell recovery percentage after thawing (50-70%) [64,147] compared to other livestock species. The most widely practiced mammalian spermatozoa cryopreservation methods still consist of a series of steps such as hypertonic cryoprotectant (CPA) addition, cooling, warming and CPA removal [158]. During this freezing process the sperm membranes undergo major detrimental physical stressors. The minimization of those stressors has not yet been successfully accomplished [147]. In order to improve methods of semen cryopreservation it is essential to better understand the physical changes that sperm experience under freezing procedures [158]. Moreover, it is clear from the variable percentages of cell viability after thawing, that substantial differences in sperm cryobiological properties exist among species. For this reason it is also particularly important to conduct species-specific studies on sperm cryo-injuries in order to design appropriate semen freezing protocol for each species [158].
Cryopreserved oocytes and embryos provide the opportunity to overcome the difficulties of donor recipient synchronization during SOV and ET. Maturation, fertilization and embryo development of cryopreserved oocytes has been achieved in a number of species [64]. The feasibility of the technique has been demonstrated by the birth of live animals using cryopreserved oocytes [91,106,133]. Unfortunately, the cryopreservation of ova is not yet particularly successful even if some progress has been made in the last 15 years. The problem seems to lie at the organelle/subcellular level. Cooling oocytes to low temperatures damages the plasma membrane, cytoskeleton, and cortical granules with consequent cell death [146]. More recent techniques of ultra-rapid cooling, such as the method proposed by Vajta of the open pulled straw (OPS), seem to have had some success [143].

Cryopreservation of embryos of many mammals has achieved acceptable rates of success. The birth of live offspring from cryopreserved embryos is possible for many species [21,132]. In bovine, cryopreservation of embryos is highly successful with both slow freezing and vitrification protocols [158]. However, pregnancy rates with fresh embryos are still significantly higher than after cryopreservation [59]. In pig embryos the high lipid content makes the freezing process more complicated [64].

There is an increasing interest in the development of technologies for isolating and cryopreserving the earliest stage of male and female reproductive cells as well as the testicular and ovarian tissues. In males, germ cells from both more advanced stages (spermatidis and spermatozoa) and earlier stages (spermatogonia) have been successful retrieved from cryopreserved testicular tissue [3-4]. These germ cells, as well as somatic cells from cryopreserved testicular tissue, can be used to restore fertility in humans [4] and animal species. The cryopreservation and transplantation of testicular tissue in toto is more challenging because of the many different cell populations (i.e., Leyding, Sertoli cells, and germ cells) with varying cellular biophysical properties [158].

Ovarian tissue cryopreservation also holds an attractive promise for both human and animal reproduction. Cryopreserved ovarian tissue can be transplanted to restore fertility or can be used for in vitro maturation of primordial follicles [54,103]. Using CPA such as Me2SO or ethylene glycol it was possible to cryopreserve ovarian tissue from various mammals [1]. However, the large ovarian size of many mammals makes the cryopreservation of the ovarian tissue difficult. Moreover, the ova increase in size during the process of oogenesis and thus accumulate a large quantity of water that can potentially damage the cells during the freezing process [158]. Future research should focus on incremental improvements to enable the survival of oocytes in more intermediate-advanced stages.

Gender selected semen

For many years livestock owners have desired a method to predetermine the sex of the offspring. In the case of the dairy cattle industry the desire is to produce heifers that will contribute to the lactating herd. Gender selected semen can be used during AI [98,125] and during IVEP [23,90]. In the latter case, the number of oocytes that can be fertilized is greatly amplified [56]. Semen can be sorted into X and Y-chromosome-bearing fractions with an accuracy greater than 90% using the flow cytometric technique [156]. Moreover, since the 1980s the flow cytometric sorting speed has increased from 350,000 sperm/h to 15-20 million sperm/h [159]. However, the sorting speed and inconsistent pregnancy rates still remain limiting factors for a widespread application of this technology in the traditional AI programs. Alternatively, IVF can highly increase the fertilization efficiency of the sorted semen as a lower number of sperm per oocyte is required [159]. In this regard, many aspects of in vitro fertilization and subsequent embryo culture have been intensely investigated to improve the fertilization rates with sexed semen and many others are needed to optimize the system [reviewed in 159]. To date sexed semen has some commercial application only in bovine. In pigs the large amount of sperm needed for AI and the high sensitivity of the sorted sperm to cryopreservation are still limiting factors for the commercially use of sorted boar semen [114]. The commercial use of sorted stallion semen depends on the consistency of the fertility after sorting and freezing as this species presents very high variability among animals and ejaculates [114]. In the sheep, numerous advancements in the AI and ET techniques can be expected to enhance the rate of commercial application of sexed selected semen [114].

New discoveries open up new possibilities

In vitro produced embryos present many chromosomal, gene expression and metabolic aberrations [87,110]. One of the major limitations for improvement of ART is the poor knowledge of germ cell and embryo biology. The study of the secretome in conjunction with genomics, proteomics and metabolomics approaches can give a more
quantitative indication of cellular and embryonic function, providing means to improve the IVEP. Several “omics”
technologies have been applied, but at present, very little is known about germ cell and embryo protein production.
Recently, proteomic studies have been undertaken to study protein composition in mammalian embryos and results
from such an approach would likely allow for improvement in IVEP [77]. The evaluation of IVEP efficiency is currently
based on parameters such as cleavage and embryo development rates, and the morphological appearance of the
germs cells and embryos. These criteria have proven to be insufficient as resulting pregnancy rates from IVEP are still
only about 50%. Composition of the medium and environmental conditions can have a deep impact on IVEP efficiency.

Very recently extraordinary discoveries in the field of stem cell biology have made the future of the ART
particularly exciting. In recent studies, putative germ cells, oocytes and spermatozoa have been derived from embryonic
stem cells (ESC), and somatic stem cells. Hubner et al. [69] were able to differentiate in vitro ESC into primordial
germs cells (PGC) that after further culture became morphologically similar to oocytes, including the expression of
genres associated with the zona pellucida. In another study, it has been demonstrated that mouse ESC were able to
participate in spermatogenesis when transplanted into reconstituted testicular tubules in vitro [141]. In 2006, a striking
paper reported the case of skin fetal stem cells expressing several germ-specific markers in vitro. After 30-40 days of
culture cells appeared to look like oocytes surrounded by cumulus cells [35]. Lately, the dogma that oogenesis is
completed before or soon after birth, and so the number of oocytes is predetermined at birth, has been challenged.
Tilly and his group have demonstrated oocyte regeneration from presumed germ cells in bone marrow and peripheral
blood [104]. Other authors have shown a return of fertility after autologous stem cell transplantation [63]. The possibility
of using these different types of stem cells (ESC, somatic, and bone marrow stem cells) is exciting and promising for
the future of reproduction. In humans, a number of couples struggling with infertility could be helped, while stem cells
from livestock animals could help to restore fertility of animals of high genetic merit whose reproductive function was
compromised. However, many are the advancements necessary before clinical application of these cells can take
place.

Many micro-manipulation procedures, such as nuclear transfer, embryo splitting, pronuclear injection, embryo
biopsy, embryo aggregation (for chimera production) and intra-cytoplasmic sperm injection, have been developed for
oocytes, embryos or sperm. These technologies need to be considered when discussing the ART portfolio. These
technologies have been described in numerous other publications [84,123-124,157] and will not be covered in this
review.

Assisted reproductive procedures and methods have developed rapidly in recent years. However, the
technology and equipment of assisted reproduction has not followed a similar transformation. As examples, semen is
still processed in test tubes in the IVF routine; oocytes maturation, fertilization and culture still occur in culture dishes
with large volume of media, and oocytes/embryo manipulation is performed manually. Microfluidics and miniaturized
devices represent a significant revolutionary technology for IVF. Currently two techniques are used to fractionate the
good motile sperm for in vitro fertilization: the swim up method and the gradient separation. Some studies have
demonstrated that these techniques may damage the DNA and produce oxygen free radicals [2,161]. However,
microdevices with parallel laminar flow can be a simple atraumatic method to separate motile sperm [136]. Beebe et
al. [8] reported a higher cleavage percentage of pig oocytes matured in microchannels versus the controls (500 ml
culture dishes). This same group developed a successful microdevice for the gentle suction of the oocytes cumulus
cells [8]. Fertilization of pig oocytes with an integrated polydimethylsiloxane (PDMS) microfluidic device was also
conducted with success [136]. Insemination in microdevices reduces the number of sperm needed and more closely
resembles the in vivo situation where only a few hundred sperm eventually reach the ampulla for fertilization [127].
Microfluidics devices have enhanced the development of mouse and porcine embryos [136]. In vivo, the embryo is in
a continuous changing environment from the site of fertilization through the length of the oviduct and into the uterus.
Traditionally embryo culture systems utilize one medium from the time of insemination to the embryo transfer. The
volume of medium used is huge compared to the in vivo situation and this may decrease the presence of embryotrophic
factors [136]. Moreover, embryos must be handled during media changes. Microfluidic devices provide the embryos
with a microenvironment more like that in vivo. Medium in the device can be changed gradually, without manipulation
of the embryos. Moreover, the microfluidic system can allow the integration of all IVEP steps [136]. Development of
an integrated system, where each step follows the other without cell manipulation, can result in improved efficiency of
the IVEP [79].
IV. GENETICALLY MODIFIED LIVESTOCK

The production of transgenics provides methods to rapidly introduce “new” or modified genes and DNA fragments into livestock without crossbreeding. It is a more precise technique, but not fundamentally different from genetic selection or crossbreeding in its result. Much has been written about the methodologies used to produce transgenic livestock [149] and that aspect will not be covered in this review. The obvious question is “WHY GENETICALLY MODIFY LIVESTOCK?” The answer is not so straightforward; however, some of the reasons are to: 1) study the genetic control of physiological systems, 2) build genetic disease models, 3) improve animal production traits, and 4) produce new animal products. This question will continue to be debated, refined and considered well into the future.

The focus of this section will be on improvement of animal production traits and production of new animal products. There are many potential applications of transgenic methodology to develop new and improved strains of livestock. Practical applications of transgenics in livestock production include enhanced prolificacy and reproductive performance, increased feed utilization and growth rate, improved carcass composition, improved milk production and/or composition, modification of hair or fiber and increased disease resistance. Development of transgenic farm animals will allow more flexibility in direct genetic manipulation of livestock. Gene transfer is a beneficial way of altering the genome of domestic livestock. The use of these tools will have a great impact toward improving the efficiency of livestock production and animal agriculture.

Applications of Transgenic Animals in Agriculture

There are numerous potential applications of transgenic technologies to develop new or altered strains of agriculturally important livestock. Practical applications of transgenics in livestock production include improved milk production and composition, increased growth rate, improved feed utilization, improved carcass composition, increased disease resistance, enhanced reproductive performance, increased prolificacy, and altered cell and tissue characteristics for biomedical research and manufacturing [155]. The production of transgenic swine with human growth hormone (GH) serves as an excellent example of the value of this technology [55]. Transgenic alteration of milk composition has the potential to enhance the production of certain proteins and/or growth factors deficient in milk [15]. The improvement of the nutrient or therapeutic value of milk may have a profound impact on survival and growth of newborn in both humans and animals. Additionally, other animal products, such as eggs and meat could benefit from the use of transgenesis. Genes could be targeted that could result in continuous egg production in chickens, and combat reproductive senescence not only in chickens but in other species as a result of physiologic events such as lactation, anorexia, poor nutrition and season of the year.

The application of transgenics already is being utilized by commercial aquaculture to enhance the growth of commercially valuable fish. Fish embryos have been microinjected with a DNA construct containing either bovine or Chinook salmon GH. These transgenic approaches also would lend themselves to improvement of the nutritional value of fish. For example, enhancing the omega-3 fatty acid in fish consumed by humans may contribute to a decreased the occurrence of coronary heart disease. In fact, transgenic pigs have been produced which contain elevated levels of omega-3 fatty acids [80]. Transgenic technology could provide a method to transfer nutritionally beneficial traits to other animal derived food sources.

Modification of Milk

Advances in recombinant DNA technology have provided the opportunity either to change the composition of milk or to produce entirely novel proteins in milk. These changes may add value to, as well as increase, the potential uses of milk.

The improvement of livestock growth or survivability through the modification of milk composition involves production of transgenic animals that: 1) produce a greater quantity of milk; 2) produce milk of higher nutrient content; or 3) produce milk that contains a beneficial “nutriceutical” protein. The major nutrients in milk are protein, fat and lactose. By elevating any of these components, we can impact growth and health of the developing offspring. In many production species such as cattle, sheep and goats, the nutrients available to the young may not be limiting. However, milk production in the sow limits piglet growth and therefore pig production [57]. Methods that increase the growth of piglets during suckling result in increased weaning weights [102], decreased days required to reach market weight, and thus decreased feed requirements for the animals to reach market weight.
Cattle, sheep and goats used for meat production may also benefit from increased milk yield or composition. In tropical climates, *Bos indicus* cattle breeds do not produce copious quantities of milk. Improvement in milk yield by as little as 2–4 liters per day may have a profound effect on weaning weights in cattle such as the Nelore breed in Brazil (Figure 4). Similar comparisons can be made with improving weaning weights in meat type breeds like the Texel sheep and Boer goat. This application of transgenic technology could lead to improved growth and survival of offspring.

![Cattle and sheep](image)

**Figure 4.** Can transgenesis produce similar milk yields? Even small improvements in milk yield of Nelore cows (left) using genetic material from high producing Holsteins (right) could have a large impact on beef production in Brazil.

A second mechanism by which the alteration of milk composition may affect animal growth is the addition or supplementation of beneficial hormones, growth factors or bioactive factors to the milk through the use of transgenic animals. It has been suggested that bioactive substances in milk possess important functions in the neonate with regard to regulation of growth, development and maturation of the gut, immune system and endocrine organs [52]. Transgenic alteration of milk composition has the potential to enhance the production of certain proteins and/or growth factors that are deficient in milk [150]. The over expression of a number of these proteins in milk through the use of transgenic animals may improve growth, development, health and survivability of the developing offspring. Some factors that have been suggested to have important biological functions in the neonate are obtained through milk include IGF-I, EGF, TGF-β and lactoferrin [52].

Other properties of milk that are considered for modifications are those that affect human and animal health. It has been shown that the preformed specific antibodies can be produced in transgenic animals [135]. It should be possible to produce antibodies in the mammary gland that are capable of preventing a mastitis infection in cattle, sheep and goats and MMA (mastitis-metritis-agalactia) in pigs, and/or antibodies that aid in the prevention of domestic animal or human diseases [155]. Another example is to increase proteins that have physiological roles within the mammary gland itself such as lysozyme [92] or other anti-microbial peptides.

The overall result of the transgenic modification of milk will be the creation of more uses of milk and milk products in both agriculture and medicine [5,38,102,144]. This is truly a “value-added” opportunity for animal agriculture by increasing the concentrations of existing proteins or producing entirely new proteins in milk.
Modification of Growth and Carcass Composition

The production of transgenic livestock has been instrumental in providing new insights into the mechanisms of gene action implicated in the control of growth [36-37,101,112-113,115,148]. It is possible to manipulate growth factors, growth factor receptors and growth modulators through the use of transgenic technology. Transgenic sheep and pigs have been used to examine postnatal growth of mammals. GH and IGF genes have been incorporated and expressed at various levels in transgenic animals [122]. Transgenic livestock, as well as salmon and catfish, have been produced which contain an exogenous GH gene. This type of work enabled the study of chronic expression of these hormones on growth in mammals and fish. Results from one study have shown that an increase in porcine GH leads to enhancement of growth and feed efficiency in pigs [148]. In fish, increased GH levels have lead to dramatic (30–40%) increase in growth rates in catfish by introducing salmon GH into these animals [33]. Introduction of salmonid GH constructs has resulted in a 5–11-fold increase in weight after 1 year of growth [28-29,33]. This illustrates the point that increased growth rate and ultimately increased protein production per animal can be achieved via transgenic methodology.

The Rendement Napole (RN) or Acid-Meat gene has been implicated in lower processing yields in lines of Hampshire and Hampshire crossbred pigs. “Knocking-out” the RN gene may provide a method to alter post-mortem pH, and, thereby, increase meat tenderness. Other specific loci which may affect growth patterns are the ryanodine receptor, the myo-D [58,131], GH releasing factor, high affinity IGF binding proteins (IGFBP-1 to IGFBP-6), the sheep callipyge [130] and the myostatin (growth/differentiation factor-8, GDF-8) [94] genes. Based on a recent report in the mouse [94], the myostatin gene is an exceptionally intriguing potential locus to “knocking-out” in meat producing species. The loss of the myostatin protein results in an increase in lean muscle mass. Certainly, there are numerous potential genes related to growth, including growth factors, receptors or modulators which have not been used, but may be of practical importance in producing transgenic livestock with increased growth rates and/or feed efficiencies.

Another aspect of manipulating carcass composition is that of altering the fat or cholesterol composition of the carcass. By altering the metabolism or uptake of cholesterol and/or fatty acids, the content of fat and cholesterol of meats, eggs and cheeses could be lowered. There is also the possibility of introducing beneficial fats such as the omega-3 fatty acids from fish or other animals into our livestock [80]. In addition, receptors such as the low-density lipoprotein (LDL) receptor gene and hormones like leptin are potential targets that would decrease fat and cholesterol in animal products.

The use of transgenic technology to modify feed efficiency and/or appetite could profoundly impact livestock production. Again, increased uptake of nutrients in the digestive tract, by alteration of the enzyme profiles in the gut, could increase feed efficiency. The ability to introduce enzymes such as phytase or xylanase into the gut of species such as swine or poultry where it is not normally present is particularly attractive. The introduction of phytase would increase the bioavailability of phosphorus from phytic acid in corn and soy products. Golovan et al. [49] reported the production of transgenic pigs expressing salivary phytase as early as 7 days of age. The salivary phytase provided essentially complete digestion of the dietary phytate phosphorus in addition to reducing phosphorus output by up to 75%. Furthermore, transgenic pigs required almost no inorganic phosphorus supplementation to the diet to achieve normal growth. The use of phytase transgenic pigs in commercial pork production could result in decreased environmental phosphorus pollution from livestock operations.

Modification of Disease Resistance

An interesting aspect of agricultural transgenics is the potential to increase disease resistance by introducing specific genes into livestock. Identification of single genes in the major histocompatibility complex (MHC) which influences the immune response, was instrumental in the recognition of the genetic basis of disease resistance and susceptibility [9]. The application of transgenic methodology to specific aspects of the immune system should provide opportunities to genetically engineer livestock with superior disease resistance.

It has only been realized recently that there are many aspects of disease resistance or susceptibility in livestock that are genetically determined [82]. One specific example where transgenesis has been applied to disease resistance in livestock is the attempt to produce cattle resistant to mastitis. Mastitis is an inflammation of the mammary gland, typically caused by infectious pathogen. Mastitis causes decreased milk production. Further, treatment and prevention of mastitis is costly both monetarily and in increased labor. Transgenic dairy cows that secrete lysostaphin into their milk have been produced to address the mastitis issue. Lysostaphin is an antimicrobial peptide
that protects mammary gland against *Staphylococcus Aureus* infection by killing the bacteria in a dose-dependent manner [31]. *Staphylococcus Aureus* is a major mastitis-causing pathogen.

The application of nuclear-transfer technology will enable the augmentation of beneficial alleles and/or the removal (via gene "knock-out") of undesirable alleles associated with disease resistance or susceptibility. An example is "knocking-out" the intestinal receptor for the K88 antigen. The absence of the antigen has been shown to confer resistance to both experimentally and naturally induced infection of K88-positive *E. coli* [39]. Potential areas of investigation include resistance to: 1) parasitic organisms such as trypanosomes and nematodes, 2) viral or bacterial organisms such as bovine leukemia virus, pseudorabies virus, foot and mouth virus, clostridium and streptococcus, and 3) genetic diseases such as deficiency of UMP synthase (DUMPS), mule foot and bovine leukocyte adhesion deficiency (BLAD).

The opportunity to produce animals that could self-immunize against pathogens is an exciting application of transgenic technology. The design of transgenes that would be expressed in response to specific stimuli or physiological state could produce antigens that result in immunization of the transgenic animal to that particular disease. Transgenes will be designed which respond to specific stimuli like feeding zinc or a specific antibiotic to produce antigens that could raise protective antibody titers.

In the future, we may be able to produce prion-free, scrapie-free and BSE-free livestock using the genetics from naturally resistant animals in cloning schemes. An example of this application is the production of transgenic mice expressing either the human or bovine prion protein. Each of these mouse strains was inoculated with the prions that cause bovine spongiform encephalopathy (BSE) or with a variant of Creutzfeldt-Jakob disease (vCJD). BSE was transmitted to the mice containing the bovine prion protein but was not transmitted to transgenic mice containing the human prion protein [13]. However, all three transgenic mouse lines containing the human prion protein showed transmission of the disease when inoculated with vCJD [13]. Another example of this potential application is the production of fetuses that are resistant to brucellosis [129]. This is only a partial list of organisms or genetic diseases that decrease production efficiency and may also be targets for manipulation via transgenic methodologies.

**Modification of Reproductive Performance and Prolificacy**

Several potential genes have recently been identified which may profoundly affect reproductive performance and prolificacy. These include the estrogen receptor (ESR) and the Boroola fecundity (FecB) genes. Rothschild et al. [118] have reported an association of a polymorphism in the ESR gene with litter size in pigs. They found a difference of 1.4 more pigs born per litter between the two homozygous genotypes. Introduction of a mutated or polymorphic ESR gene could increase litter size in a number of diverse breeds of pigs. A single major autosomal gene for fecundity, the FecB gene, which allows for increased ovulation rate, has been identified in Merino sheep [108]. Each copy of the gene has been shown to increase ovulation rate by approximately 1.5 ova, although the increase in litter size is not completely additive [108]. Production of transgenic sheep containing the appropriate FecB allele could increase fecundity in a number of diverse breeds. Identification of additional genes involved in prolificacy and fecundity from hyperprolific breeds/strains of swine (Meishan), sheep (Finnish Landrace) and cattle (high twinning) will provide additional opportunities to increase reproductive performance. The manipulation of reproductive processes using transgenic methodologies is only beginning and should be a rich area for investigation in the future.

**Modification of Hair and Fiber**

The control of the quality, color, yield and even ease of harvest of hair, wool and fiber for fabric and yarn production has been an area of focus for transgenic manipulation in livestock. The manipulation of the quality, length, fineness and crimp of the wool and hair fiber from sheep and goats has been examined using transgenic methods [66,111]. Increasing the elasticity of the fiber and increasing the fiber strength also may lend themselves to transgenic modification [7]. In the future transgenic manipulation of wool will focus on the surface of the fibers. Decreasing the surface interaction could decrease shrinkage of garments made from such fibers.

A novel approach to produce useful fiber has been recently accomplished using the milk of transgenic goats [76]. Spiders that produce orb-webs synthesize as many as seven different types of silk used in making these webs. Each of these silks has very specialized mechanical properties that make them distinct from other synthetic and natural fibers [76]. One of the most durable varieties is dragline silk. This material can be elongated up to 35% and has tensile properties close to those of the synthetic fiber Kevlar™. This silk has the energy absorbing capabilities
before snapping which exceeds those of steel. The protein monomers that assemble to produce these spider silk fibers have been produced in the milk of transgenic goats. The numerous potential applications of these fibers include medical devices, suture, ballistic protection, aircraft, automotive composites and clothing to name a few.

V. STEM CELLS AND FUTURE TECHNOLOGIES

In mammals, stem cells are defined as a unique cell population characterized by nearly unlimited self-renewal and capacity to differentiate via progenitor cells into terminally differentiated somatic cells. Stem cells may be of embryonic or adult origin. Adult stem cells are located in many specialized tissues, including the liver, skin, brain, fat, bone marrow, and muscle. As a result of stem cell activity, adult tissues are continuously renewed, even in the absence of injury, to ensure maintenance of the cell type throughout the life of the animal. Pluripotency in mammals is restricted to the zygote, early embryonic cells, primordial germ cells, and the stem cells derived from embryonic carcinomas. Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst. In contrast to adult stem cells, ES cells are pluripotent, contribute to all three primary germ layers (endoderm, mesoderm, and ectoderm), indefinitely proliferate, and maintain an undifferentiated phenotype. Embryonic germ (EG) cells are derived from primordial germ cells, which are progenitor cells of the sperm and egg in the adult animal. EG cells reintroduced to the early embryo are capable, like ES cells, of colonizing fetal cell lineages and possess the ability to differentiate in vitro to a variety of cell types. The potential of stem-cell technology makes it a valuable and exciting science. Adult and embryonic stem cells may possess the ability to restore or replace tissue that has been damaged by disease or injury. Pluripotent, in vitro cell lines offer an opportunity to study the early stages of embryonic development not accessible in utero and provide a powerful tool to facilitate genetic modification of animal genomes.

Embryonic Stem Cells, iPSC Cells, Embryonic Germ Cells

The early mammalian embryo is composed of cells that have the potential to contribute to all tissue types in the body, a property termed pluripotency. As the embryo develops to the blastocyst stage, it forms an outer cell layer and an inner cluster of cells referred to as the inner cell mass (ICM). The outer cells become the trophectoderm and ultimately the placenta. The ICM cells create all tissues in the body, as well as non-trophoblastic structures that support the embryo. Embryonic stem cells are derived in vitro from the ICM. ES cells were first successfully developed from mouse blastocysts in 1981[41,93]. ES cells contribute to all three germ layers in the developing fetus, proving that they are pluripotent, but ES cells fail to contribute to the trophectoderm, revealing that they are not totipotent. ES cells, when removed from feeder layers in culture, begin to differentiate into multilayered differentiated structures called embryoid bodies. In addition to blastocyst injection, the in vivo developmental potential of ES cells can be tested by injecting ES cells into severe combined immunodeficient (SCID) mice [44]. Benign teratomas form where the cells are injected and contain tumors representing all three germ layers. ES cells derived from human embryos have also been reported [140].

In 2006, Takahashi and Yamanaka first established a technique by which differentiated mouse cells can be reprogrammed into pluripotent embryonic stem cell-like cells [160]. Such induced pluripotent stem (iPS) cells were generated by retroviral-delivery of a set of 4 transcription factors, Pou5f1 (Oct3/4), Sox2, Klf4 and c-Myc, which have been known to play essential roles in self-renewal and pluripotency of embryonic stem cells [137]. A year later, generation of iPS cells was also achieved with human cells. Thus, in theory, this technology will allow us to generate custom-made replacement tissues using autologous cells. However, because expression of Klf4 and c-Myc and the integration of retroviruses into particular genomic sites may initiate tumorigenic transformation of normal cells, the original method has been modified to overcome such safety issues. So far, the modifications made include the use of 1) only two factors (Oct3/4 and Sox2) with a chemical compound (valproic acid, an inhibitor of histone deacetylase), 2) only one factor (Oct3/4) introduced in neural stem cells that express Sox2 and c-Myc endogenously, and 3) other gene delivery methods include adenoviruses, episomes, plasmids, which will not be stably integrated into the genome, and transposons, which can be removed by transient expression of the enzyme transposase. However, the reprogramming efficiency with this method is about 1% or less, which is lower than that of the somatic cell nuclear transfer technique. Recently, it has been demonstrated that the tumor suppressor p53, which safeguards integrity of the genome, is the gatekeeper for the reprogramming [67]. Thus, transient downregulation of p53 may increase the efficiency of generating iPS cells, while raising the risk of generating tumorigenic cells. Further basic studies to
understand the mechanisms of self-renewal and pluripotency as well as tumorigenesis will eventually make iPS cells safer for practical therapeutic use.

Pluripotent embryonic germ cells can be isolated from the genital ridge of the developing mammalian fetus. EG cells closely resemble ES cell lines in the morphology of colonies, response to induced differentiation, and ability to create chimeric offspring. ES and EG cells are not the same in all respects. Differences exist in the conditions required for their isolation, culture, lifespan \textit{in vitro}, and differentiation capacity. An important difference is the genetic modifications that occur in the DNA of primordial germ (PG) cells that result in erasure of genomic imprints. The DNA modifications that occur can compromise the developmental potential of the EG cells.

Agricultural Applications for ES and EG Cells

The establishment of ES and/or EG cells from a wide variety of species will allow more flexibility in direct genetic manipulation of livestock as well as agricultural, gene-regulation, and developmental biology research. The use of ES cells in mouse developmental biology research is well documented. However, the production of a chimeric livestock species (swine and cattle) produced from ES cells has only recently been reported [20,119,154]. The use of ES or EG cells for the production of transgenic animals from DNA-transformed, individually derived and screened embryonic cell lines could allow large numbers of genetically identical animals to be established. There are many potential applications of stem cell-mediated transgenesis to develop new and improved strains of livestock.

Stem cells have revolutionized many areas of biology, and with continued research more information will be learned regarding these unique cells. Comparisons of prospective applications between mammalian adult, ES, EG, EC and iPS cells indicate varying levels of potential. Adult stem cell potential has not been as extensively investigated compared to ES cells due to the difficulty of identifying adult stem cells in a population of cells. EG cells have limited ability to recapitulate normal development due to genetic modifications affecting imprinting status of the cells. EC cells contain karyotypic abnormalities and have limited potential to transmit through the germ line of chimeric animals. ES cells do not possess any major limiting characteristics in comparison to the other cell types, which demonstrates their preferential use in scientific studies. ES cells offer a multitude of applications including access to a population of precursor cells difficult to identify \textit{in vivo}, ability to identify novel genes during early embryonic development and differentiation processes, use as a standardized \textit{in vitro} model to test embryotoxic effects of chemicals [78], the study of targeted mutations of genes that may be lethal \textit{in vivo} but can be studied \textit{in vitro}, and less expensive teratogen testing that does not involve isolating embryos or sacrificing animals. The potential applications of stem-cell technology in livestock production are great.

VI. FUTURE PROSPECTS: ARE WE GOING TO HIT HOLISM, FINALLY?

One can envisage a future where all the “omics” technologies, together with yet-to-be-developed “omics” tools, will be analyzed simultaneously in the same sample in order to provide all levels of information, from the functional (e.g., proteomics, metabolomics) to the mechanistic (e.g., genomics, CHIP-ChIP, miRNA) to the hereditable (e.g., SNP, epigenomics) understanding of the system. Before such a scenario can be reached, many technical and computational issues will need to be addressed. The identification of genes that influence and are directly responsible for economically important agricultural traits will develop rapidly now that the genome sequences for livestock species are being completed. The refinement of assisted reproductive technologies (AI, ET, IVEP) for all agriculturally important species will be necessary to realize the full benefits from the genetic information that is being identified for selection of superior animals. Improvements in gamete and embryo cryopreservation are essential to this effort as are refinements to \textit{in vitro} embryo production. The use of transgenic technologies to introduce single or multiple genes into existing genomes of livestock will play an increasingly larger role in the genetic development of our production livestock in the future. Addition of appropriate stem cell technologies to the genetic “toolbox” will further increase our capabilities to enhance and modify livestock genomes and physiology.

Until recently the approach to human knowledge, particularly to science (including livestock science), has been driven by reductionism, which has provided great insights into biology but also has not allowed for a full view of reality. Today, new technologies are at hand which allow and strongly push for a holistic approach to biology. This approach allows us to envision a future where the networks of feedback between technologies (both applied and research, Figure 5) will be not only possible but necessary in order to improve livestock production, both from a
quantitative as well as a qualitative point of view. The realization of these potential improvements of our production livestock will also require investments in technological expertise, education and animal resources. Societies and especially countries that possess or are willing to make such investments will be on the leading edge of development of genetically superior livestock for food production as we continue through the 21st century.

Figure 5. Networks between technologies need to be fully exploited in order to effectively improve livestock production. Stem cell biology, study and discovering of QTL, effective production of transgenic animals, and improvement of ART can greatly benefit from a systems biology approach. The most efficient way to improve technologies is to follow the flow of knowledge from understanding a system (systems biology) to application of technologies, which in the past may have been confusing. The concrete impact of this flow of knowledge in livestock production is achieved through ART, production of transgenic animals, selection using QTL (and new genetic technologies such as SNP), and nutrigenomics. Stem cells can be used to assist with ART (e.g., transplantation of germ cells from a superior animal in a previously sterilized animal), for production of transgenic animals, or for tissue regeneration. Subsequently, effective production level improvement can be further investigated using systems biology in order to improve the efficiency of the flow of knowledge.

VII. CONCLUSIONS

The potential applications of biotechnology in livestock production are endless. The utility of biotechnology in livestock production is limited only by our knowledge of the genes involved, gene function and gene product interactions. The development of useful biotechnology tools continues. Procedures and policies for the evaluation of the risk, food safety, efficacy and consumer benefit of products produced by these technologies need to be developed, discussed and implemented. While researchers can develop many potentially useful products using biotechnology, the promise for the consumer and society will not be realized unless we develop strategies, guidelines and regulations to get animals and their products, which have been produced by biotechnology, safely and efficiently into the marketplace.
REFERENCES


Production: toward holism.


