

EVOLUTION OF LIVESTOCK IMPROVEMENT

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Summary

Since the first domestication of livestock, people have been attempting to alter specific traits in animals that we deem to be desirable for various reasons. In doing, so we have been indirectly altering the genetic makeup of these species by selecting and breeding animals with specific traits, and therefore, particular genetic make-ups related to these traits. This initial form of genetic improvement was carried out with little or no knowledge of the underlying genetics mechanisms. However, the advent of molecular biology and a growing ability to isolate and manipulate genes has resulted in more direct approaches to genetic manipulation and selection. This encyclopaedia chapter discusses important events that are related to the area of livestock improvement including: quantitative genetic; molecular biology; reproductive technologies; transgenics; and the emerging area of livestock genomics.

1. Introduction

"As the earth and ocean were probably peopled with vegetable products long before the existence of animals; and many families of these animals long before other families of them, shall we conjecture that one and the same kind of living filaments is and has been the cause of all organic life," This profound statement was composed by the grandfather one of the most influential biologists of all time, Charles Darwin. It is amazing to think that without any knowledge of deoxyribonucleic acid (DNA) or genes as we know and understand them, Darwin in his book *Origin of the Species* (1859) and his contemporary

Gregor Mendel in his short monograph *Versuche über Pflanzen-Hybriden* (1865) (English translation *Experiments with Plant Hybrids*) were able to formulate complex and accurate concepts regarding the heritability of traits and phenotypes in living organisms. Darwin, during his travels aboard the sailing ship the *H.M.S. Beagle* made observations regarding phenotypic variations among species and their environments which lead him to his theories on the subjects of inheritance, adaptability and evolution. While Mendel, working in his garden laboratory, conducted cross-breeding experiments with pea plants and performed extensive calculations to develop his Laws of Heredity, which are still respected today. Although these two researchers approached their work in different ways, the basis of their science was the same. Both identified the fact that certain heritable phenotypes can be selected for or against. Whether this selection is by the environment and natural selection as Darwin described or by human intervention in the case of Mendel's experiments, the concept holds true. Therefore, without knowing that these phenotypes were actually based on genes, they demonstrated that the genetic make-up of living organisms can be manipulated. However, these concepts were so novel and revolutionary that it took decades for the rest of the scientific community to grasp and eventually accept them. Despite the initial resistance to their ideas, the theories proposed by these pioneers became the foundation of genetic research in livestock.

2. Livestock Improvement Techniques

2.1 Quantitative Genetics

In the early 1900's one area that embraced and applied these ideas was that of agriculture and those involved in livestock improvement by selective breeding. Using these theories, animal breeding soon became an art, with the laws of heredity, selection and breeding being used to shape the form of animals. As the benefits of this art became recognised, the science of animal breeding quickly developed with active studies conducted and theoretical explanations developed to quantify, enhance and maintain desirable characteristics. This work advanced with varying degrees of success, until the development of standardised selection criteria or selection indices intended to better rank animals genetically and identify which individuals merited breeding. This stimulated data collection on individuals and their relatives, as well as the development of procedures to estimate heritability along with genetic and phenotypic correlation and phenotypic variation based on this information. The relatively recent availability of computer technology has facilitated the development and widespread application of livestock improvement tools such as the Best Linear Unbiased Prediction (BLUP) system, which requires complex calculations, but provides highly dependable information for selection purposes. Use of such selection systems has greatly enhanced production characteristics in livestock such as swine in which growth rates, feed efficiency and percentage of carcass lean have increased dramatically in recent decades.

2.2 Reproductive Technologies

Advances in quantitative genetics have coincided with developments in reproductive techniques, which have contributed greatly to livestock improvement efforts. The earliest forms of these technologies were in the areas of artificial insemination (AI) and

embryo transfer (ET). AI and ET allow for the rapid dissemination of valuable genetic material from superior sires and dames. AI is particularly efficient as a few superior males can produce enough spermatozoa to inseminate thousands of females per year. Although ET is less efficient, as females can only produce a fraction of the gametes a male animal can, it still provides a means to improve the reproductive efficiency of genetically proven females. The history of AI dates back to the 17th century, but extensive studies and application of AI in livestock occurred in the 1900's. While early embryo transfer experiments were performed with rabbits in the late 1800's, it was 50 years before this technology was successfully applied in farm animals.

The long term storage of sperm and embryos through cryopreservation further enhanced the advantages of these techniques as cryopreservation facilitates the transport and banking of these genetics resources. These techniques and particularly AI with cryopreserved sperm have had a dramatic effect on the genetic make-up of domestic animals. This is particularly true for the dairy industry in which the vast majority of milk producing cows have descended from a select pool of genetically proven superior males. The use of frozen semen in some species, particularly swine, has been limited by the poor survival of porcine semen; however the ready availability of fresh semen from genetically superior boars has prompted the widespread use of AI in swine.

2.3 Molecular Biology

The 1953 publications by Watson and Crick opened the door to genetic manipulation as we know it today. Prior to this, it was known that a set of factors, referred to as genes, were responsible for the transmission of heritable characteristics from generation to generation. It was also assumed that genes were in some way associated with the chromosomes found in the nuclei of cells and studies had demonstrated that DNA or a factor related to DNA was indeed the carrier of genetic information. Yet, how exactly this information could be coded and transmitted remained a mystery until these two pioneers in the domain of molecular biology described DNA's double helical structure and proposed the fashion in which genetic information could be conserved between generations. In doing so they confirmed the existence of the single unifying genetic element, which Erasmus Darwin was alluding to in the quotation that debuts this text.

Although Watson and Crick opened the door on a new era in biology, this road has been paved with many important discoveries which has led us to where we are now in this era of biotechnology. Among these discovers was the identification of the codon-based genetic code, which was finally revealed through the work of several laboratories. Along with this knowledge came the possibility of directly altering DNA, through the discovery and application of restriction enzymes and ligases. These enzymes allow us to cut DNA of different origins into fragments and fuse them together to produce recombinant DNA. This technology coupled with the ability to reproduce recombinant DNA in bacteria, created the basic molecular biology tools required to study, understand and manipulate genetic materials. Genetic engineering of a living organism became a reality when a synthetic mammalian gene was constructed and inserted into the bacterium *Escherichia coli* which then was able to produce the somatostatin peptide that the gene encode. Since this time the initial trickle of developments in this domain has quickly grown to a torrent as new technologies allowing us to sequence DNA or amplify

it *in vitro* by polymerase chain reaction (PCR) have emerged. These revelations have made gene manipulation easier and more efficient and have been progressively used to alter various phenotypes in livestock. Initially this led to direct gene manipulation in the form of transgenics, but has more recently evolved into livestock genomics techniques in which unique genetic markers can be identified. These markers are then used in combination with traditional quantitative genetic approaches to manipulate the genetic makeup of a population of animals intended for a specific purpose.

3. Transgenics

Transgenesis refers to the production of transgenic organisms, whereby heritable functioning genes are transferred from one organism to another without regard for species barriers. Transgenic animal technology is built on the techniques and skills, which evolved in the various disciplines discussed here. The initial step involves the production of the DNA construct or transgene. Any transgene must contain a minimum of two essential DNA components: a regulatory sequence, often referred to as a promoter, and a gene coding sequence. The promoter sequence controls the tissues in which a gene will be expressed and can provide control over the timing of the expression. As for the coding sequence, it contains the information necessary for the protein of interest to be produced. The second step involves the introduction of the transgene into the genome of an organism to produce a transgenic animal where the transgene will be expressed.

It was only by combining their knowledge of basic genetics, recombinant DNA techniques and reproductive physiology that Gordon and associates were able to produce the first transgenic mice. The true potential of transgenic technology was later demonstrated in 1982 when a transgenic "super-mouse" appeared on the cover of the journal *Nature*. Since this point the introduction of foreign genetic material into the genome of an animal has proven extremely valuable in the study of numerous aspects of gene expression and the physiological roles of various factors. Furthermore, this technology offers the possibility of increasing the value and/or production ease of current economically important domestic farm animals (e.g. swine, cattle etc.). The potential of transgenic livestock is extremely diverse: growth regulation, augmentation of reproduction, production of valuable recombinant in animal bodily fluids, disease resistance and alteration of an animal's cell surface antigens for use in xenotransplantation; among others. This section briefly describes techniques that have been used to produce transgenic animals and leads to discussion on the application of transgenics in livestock.

3.1 Methods of Transgenesis

3.1.1. Pronuclear Injection of Fertilised Oocytes

The technique, which has been the most successful in the production of transgenic animals, is the microinjection of DNA into the pronucleus of fertilised oocytes and, as a result, this technique has been frequently reviewed. Although differences exist between species in the way in which certain steps are performed, the principles of transgenesis by microinjection remain the same. Briefly, donor females are superovulated, mated to

fertile males and the resulting early stage embryos collected at the pronuclear stage. One of the two pronuclei is microinjected with foreign DNA containing a gene of interest and a promoter sequence. The surviving embryos are re-implanted into recipient females. Offspring resulting from the recipient mothers are evaluated for the presence of the foreign DNA to identify transgenic animals, termed founders. Transgenic founders are then mated to produce specific transgenic lines.

The means by which the injected DNA is incorporated into the host genome is not completely understood. It has been proposed that repair enzymes in the developing embryo are activated by the injection of the unassociated DNA construct and create breaks in the host DNA which acts as an integration site. The site of insertion in the host genome is random and as a result different founders are unlikely to have the same insertion site. It is also possible that the injected DNA will be incorporated into more than one site in the host genome. These variations in insertion site and rate mean that the physiological timing and degree of expression of the foreign gene can vary enormously between transgenic founders and their lines. Generally, multiple copies of the injected DNA are incorporated into the host genome at the one cell stage, resulting in an animal in which each cell contains a copy of the foreign gene. If integration occurs at a later stage of development (i.e. 2 or 4 cell stage) resulting transgenic animals may have the gene in a portion of their cells and are termed chimeric or mosaic. Transgenic founders are bred to produce transgenic lines, which can be studied and the genotypic and phenotypic changes determined.

The manner in which the foreign gene or "transgene" is transmitted from the founder animal to the next generation is dependent on the circumstances surrounding the transgene's integration into the host genome. Optimally the injected DNA construct has been incorporated at the one cell stage and in a single position in the genome, so that transgene will be transmitted in a predictable Mendelian fashion. If the transgene has been inserted into more than one site, the rate of transmission of the gene and the phenotypes in the resulting transgenic line can be quite varied depending on the relative locations of insertions and the rate of expression of each insertion. Moreover, if the founder is chimeric, transmission of the transgene is dependent on whether the chimeric animal possesses it in all, none or some of its germ cell precursors. A chimeric founder, in which all germ cell precursors possess the transgene will produce transgenic offspring at the same rate as a non-chimeric founder, as opposed to a chimeric founder in which none possess the transgene who will produce no transgenic offspring. The rate of transgene transmission in chimeric animals for which only a portion of the germ cell precursors possess the transgene is dependent on the number of sperm or oocytes possessing the transgene that have the opportunity to form the next generations. Ultimately, transgenic offspring obtained from a chimeric founder are true transgenics with the transgene present in all cells.

In mice, the efficiency of production of transgenic founders is approximately 10-15 %. That is, 10-15 % of microinjected and transferred embryos develop to new-borns of which 25 % will be transgenic for an overall efficiency of 2.5-3.75 %. The efficiency of transgenesis in large domestic animals varies, but is generally considered to approach 1.0 %. This relatively low efficiency, coupled with the extended gestation and high maintenance costs of farm animals, makes the production of transgenic livestock time

consuming and expensive. Steps have been taken to better understand the mechanisms involved in transgene integration and expression and to improve on these factors, however these findings have yet to be fully exploited. Nevertheless, initially microinjection of fertilised oocytes was the primary technique for the production of transgenic livestock although the development of other techniques, to be discussed here, may soon make this approach obsolete.

3.1.2 Retroviral Infection

Retroviruses are unique organisms in that they use RNA as their genome rather than DNA, therefore they code genetic information from RNA to DNA. When retroviruses infect a host organism a DNA sequence (provirus) complementary to their RNA is produced in the infected cells. This provirus inserts itself into the genomic DNA of these cells, in much the same way microinjected DNA constructs insert into the genome of a fertilised oocyte. The result being, each time the cellular machinery of the infected cells is put into action to transcribe RNA, the RNA sequence corresponding to the viral DNA is produced. The resulting RNA may then be translated into viral proteins or become the genetic coding sequence for new retroviruses, which go on to further infect the host organism or other organisms. Thus, there exists in nature a system capable of efficiently introducing foreign DNA into cells.

The potential applications of retroviruses in transgenesis have been recognised and successfully applied. The first step in utilising retroviruses in the production of transgenic animals involves redesigning the viral genome. The genes of the virus are deleted and replaced with a RNA version of the DNA construct of interest. It is important that the flanking sequences of the construct are truly a portion of the viral genome, so that the virus is still capable of inserting DNA into host chromosomes efficiently. By engineering the retrovirus in this manner, when the virus infects cells, it inserts the DNA construct into the host genome rather than inserting information to produce more viruses. Furthermore, true viral infection does not occur, so there is no risk of disease or transmission to other animals.

Genetically engineered retroviruses are introduced into developing embryos to facilitate the transfer of the transgene into the animal of interest. This can be achieved by microinjecting the virus into blastocysts or embryos (under the zona pellucida, a natural viral barrier) or by incubating the virus with "zona pellucida free" embryos. The manipulated blastocysts or embryos are then introduced into recipient females to obtain offspring. Founder animals that result generally contain the transgene in only a portion of its cells (i.e. chimeric), since the retrovirus does not usually infect every cell in the developing animal it is introduced to. As in the case of chimeric animals produced by microinjection, only animals in which germ cell precursors possess the transgene will produce transgenic offspring.

The use of retroviral vectors for transgenesis is advantageous because, unlike microinjection, only a single copy of the transgene is integrated in the host genome and the virus can be introduced into embryos at various stages. Less desirable aspects of this procedure include the fact that retroviral vectors are limited in the size of genes that they can carry and the molecular biology involved in engineering a retrovirus is rather

demanding, requiring strong expertise in this area. Despite limitations, retroviruses have been used to successfully produce transgenic mice. Procedures have been described in which retroviral infection of embryos and integration of recombinant sequences has been achieved in cattle. While reference to unpublished data in which retroviral infection has been achieved in cells from sheep, pigs, goats and horses. Establishment of transgenic lines of domestic animals using retroviral vectors has yet to be reported. Furthermore, given the fact that no further reports on this subject have been forthcoming, the potential of this technology to produce transgenic livestock is questionable.

3.1.3 Spermatozoa Mediated Gene Transfer

A 1989 study prompted a great deal of interest in the concept of using motile spermatozoa as vectors to introduce foreign DNA into oocytes. In this study, the incubation of washed mouse spermatozoa, in the presence of DNA fragments, lead to the production of transgenic mice when these spermatozoa were used for *in vitro* fertilisation. However, attempts to repeat these experiments by other researchers proved to be unsuccessful, indicating that an unknown essential element present in the initial experiments was not identified. The potential value of this form of transgenesis has still proved to be enticing, since it requires neither specialized equipment nor a high level of expertise and as a result research in this area continued.

The ability of foreign DNA to enter the head of the spermatozoa has been successfully demonstrated in a wide variety of species. In the mouse and pig it was reported that approximately 30% of the washed spermatozoa incubated in the presence of foreign DNA were able to bind the DNA successfully. While uptake of DNA by washed bovine spermatozoa was found to be approximately 19 %. The feasibility of improving the association and internalisation of foreign DNA into porcine and bovine spermatozoa with the use of electroporation has also been studied. Electroporation involves the use of electrical pulses to permeate the cellular membrane and facilitate the movement of DNA into the cell. In both species it was determined that electroporation results in an increase in the absorption of foreign DNA by the spermatozoa in comparison to non-electroporated spermatozoa.

As well, it was found that the electroporated bovine spermatozoa were able to carry foreign DNA into oocytes at fertilisation. Although encouraging, these studies failed to indicate whether the identified association of DNA with spermatozoa is appropriate for the delivery, release and integration of foreign DNA in these species. However, the laboratory that first reported sperm mediated production of transgenic animals, has again published reports of the production of transgenic mice and pigs with this technique. Furthermore, a variation on this approach has recently been reported. With this procedure, sperm cells are exposed to a series of harsh treatments and co-incubated with plasmidic DNA. The sperm heads and DNA are then introduced into oocytes by intracytoplasmic sperm injection (ICSI). This technique has been quite successful in producing transgenic mice. Thus, it would appear that sperm mediated DNA transfer may well prove useful in producing transgenic livestock, but the elements necessary for its successful application must be fully identified in order to provide more consistent results.

3.1.4 Spermatogonial Transfer

A series of publications by Brinster and colleagues followed closely by an extensive commentary on this research brought attention to the potential of spermatogonial stem cell transplantation for, among other things, the production of transgenic animals. Spermatogonia form the basal layer of the seminiferous tubules of the testis and possess the potential to divide and differentiate to produce mature spermatozoa. Spermatogonia can be divided into 3 types: spermatogonial stem cells (Type A0 spermatogonia), proliferative spermatogonial stem cells (Types A1, A2, A3, A4 spermatogonia) and differentiating spermatogonia (Type B spermatogonia etc.). The spermatogonial stem cells (Type A0) are considered to be a “reserve” type of cell that repopulate the testis, if the other types are depleted due to trauma. While proliferative cells maintain a self-renewing cycle, with sequential differentiation of Type A1 to Type A4 cells. The Type A4 spermatogonia divide to give rise to new Type A1 spermatogonia, as well as differentiating spermatogonia, thereby re-initiating the cycle. It is the differentiating spermatogonia, which give rise to mature spermatozoa following a series of cell divisions and modifications.

Spermatogonial stem cells, like embryonic stem cells, are considered to be pluripotent and therefore are capable of participating in embryogenesis and contributing cells to any part of the fetus including the germ line. Yet, spermatogonial stem cells are quite unique in that they are capable of self-renewal. Brinster and colleagues have recognised the potential of being able to recover spermatogonial stem cells, genetically manipulate them *in vitro*, and transplant the cells into a recipient testis. The recipient animals would then act as vectors, producing male gametes originating from the genetically modified spermatogonia. Offspring produced with these gametes would be transgenic for the gene introduced into the male stem cells *in vitro*. This group has taken the first steps necessary in this process by successfully developing a process of transplanting testis cells from one male mouse to another as well as establishing spermatogonial cell culture and cryopreservation systems. Development of this technology in livestock to date has been limited to manipulation of the pig male stem cells *in vivo*. However, the advances in this technology with the mouse may lead the way for further research with this potentially powerful tool for producing transgenic animals in agriculture.

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Biographical Sketch

Michael K. Dyck obtained a Bachelor of Science in Agriculture Degree, with the honour of distinction, from the University of Manitoba. He pursued graduate studies at the University of Guelph where he obtained a Master of Science from the Department of Animal and Poultry Science, with a specialization in reproductive physiology. Dr. Dyck then went on to work in the field of swine reproduction and later Canada's largest human sperm banking facility, where he gained significant experience in different advanced reproductive technologies and regulatory affairs. He enrolled in Laval University's Ph.D. program where he was a Natural Science and Engineering Research Council Scholar. During his Doctoral training, Dr. Dyck conducted research into the use of transgenesis to alter commercially desirable traits in swine. The possibility of increasing reproductive and growth efficiencies, as well as using pigs as bioreactors for the production of recombinant proteins was investigated. Dr. Dyck has developed and applied numerous techniques involved in the production and physiological evaluation of transgenic mice and swine. He then joined TGN Biotech Inc., Quebec City as a Natural Science and Engineering Research Council Industrial Research Fellow and eventually assumed the position of Director of Transgenics and Cell Biology for this company. Dr. Dyck joined the Faculty of Life and Environmental Sciences at the University of Alberta in January 2004 and as member of the Swine Reproduction and Development Program has been conducting research focused on the development and application of molecular techniques and advanced reproductive technologies, in collaboration with the pork production industry, to improve reproductive efficiency in swine.