The Evolution of Farm Animal Biotechnology

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Abstract

The domestication of farm animals starting 12,000–15,000 years ago in the Middle East was a seminal achievement in human development that laid the foundation of agriculture as it is known today. Initially, domesticated animals were selected according to phenotype and/or specific traits adapted to a local climate and production system. The science-based breeding systems used today originated with the introduction of statistical methods in the sixteenth century that made possible a quantitative approach to selective breeding for specific targeted traits. Now, with the availability of accurate and reliable DNA analysis, this quantitative approach has been extended to DNA-based breeding concepts that allow a more cost-effective but still quantitative determination of a genomic breeding value (GBV) for individual animals.

The impact of these developments was dramatically enhanced with the introduction of reproductive technologies extending the genetic influence of superior individual animals. The first of these was artificial insemination (AI) that started to be developed in the late nineteenth century. Industry uptake of AI was initially slow but increased dramatically following the development of semen extenders, the reduction of venereal disease risk by inclusion of antibiotics, and most significantly the development of effective freezing and cryostorage procedures in the mid-twentieth century. AI is now used in most livestock breeding enterprises, most notably by the dairy industry where more than 90% of dairy cattle are produced through AI in countries with modern breeding structures.

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Embryo transfer (ET), a technology that for the first time allowed exploitation of the female genetic pool, was made possible through the major advances in the biological sciences in the later part of the twentieth century. Advances in understanding of the reproductive cycle and its hormonal control, the availability of purified gonadotropins, and improved cell and embryo culture procedures all played significant roles. ET is now being increasingly implemented in top end breeding endeavors, particularly in the top 1–2% of a given cattle population. But its real impact is yet to come as ET is the key enabler in the introduction of the next generation of enhanced breeding technologies. ET has already played a key role in advances such as in vitro production of embryos, sexing, cloning, and transgenesis. With the birth of "Dolly," the cloned sheep, in 1996, a century-old dogma in biology, which inferred that a differentiated cell cannot be reprogrammed into a pluripotent stage, was abolished. Today, through recent developments in molecular cell biology, available protocols are efficient enough to allow commercial application of somatic cloning in the major farm animal species. This will not only further enhance the rate of genetic gain in herds and flocks but through the recent advent of precise genome editing tools allow the production of novel germlines for agricultural and biomedical purposes through the capacity to genetically modify farm animals with targeted modifications with high efficiency. This paves the way for the introduction of the precision breeding concepts needed to respond to future challenges in animal breeding, stemming from matching the demands of ongoing hyperbolic human population growth to the limited availability of arable land and environmental constraints.

1.1 Introduction

The great variety of phenotypes presently seen in domesticated animals is the product of human-directed breeding over many centuries. Compelling evidence of domestication of livestock more than 10,000 years ago is provided by archeological findings showing that milk and dairy products were then already part of a normal human diet. Up to the last century, selection of breeding stock for specific phenotypes or production traits was made by simple observation with science-based quantitation and breeding for specific genotypes only introduced following the development and introduction of statistical methodologies in the late nineteenth century. The accurate and reliable prediction of genetic traits made possible from this introduction revolutionized breeding practices and, together with advances in DNA technology, ultimately led to the quantitative molecular genetic selection procedures used today. The next major advance was the development over the past 50 years, of a growing array of reproductive biotechnologies, most notably artificial insemination (AI) and embryo transfer (ET). The full impact emerging from linking molecular genetics and reproductive technology is yet to be realized. Already one outcome has been that it is now not only possible to precisely and reliably analyze genomes but in an equally precise and reliable way engineer the genome to both

enhance desired production traits and introduce novel production traits. Another impressive outcome of this alliance is the development of reliable cloning procedures that utilize somatic cells as the genome source, a major achievement that opens new horizons of possibilities that assures an exciting future for animal breeding enterprises. This chapter covers the cornerstones of the history of animal breeding, from its genesis many thousand years ago to today, with focus on the biotechnological advances that are and will be increasingly employed by livestock breeding enterprises to address the hyperbolical increasing human demand for conventional and novel animal products. Important milestones of this evolutionary process of animal breeding are provided in Table 1.1.

1.2 Evolution of Farm Animal Breeding

1.2.1 From Domestication to Systematic Breeding Concepts for Farm Animals

Domestication of animals was the foundation stone of agriculture as it is known today (Diamond 2002) and a key advance in human development. Classical studies on the historic pathways of domestication, primarily based on archeological evidence, are now being overwritten by a growing body of information provided by DNA studies. Analysis of mitochondrial DNA has been particularly useful in this regard as it is maternally inherited and has various properties, including the lack of recombination, high mutation rates, and the presence of multiple copies (Bradford et al. 2003; McHugh and Bradley 2001). Conjointly these disciplines provide compelling evidence that domestication started around 10.000–15.000 BC, predominantly in the Middle East (Connolly et al. 2011). Archeological findings there and on the British Isles revealed that approx. 14.000–17.000 years ago, humans already kept farm animals and that milk and dairy products were essential parts of their nutrition (Beja-Pereira et al. 2006; Larson et al. 2007). DNA studies of the two main bovine species, taurine and zebu cattle, indicate separate domestications starting ~8000 years BC in Southwestern Asia and the Indus valley (Zeder et al. 2006). The progenitor species was the aurochs (*Bos primigenius*), a tall and well-fortified animal with very long horns, the latter a feature still reflected in most current cattle breeds (Schafberg and Swalve 2015). Domestication of pigs took place independently at predominantly two locations, in East Anatolia and China (Groenen 2016), sheep and goats were domesticated in West and East Asia, and horses stem from the Eurasian steppes (Wang et al. 2014).

The rich variety of geno- and phenotypes in farm animals now extant is the product of man-made breeding over the intervening centuries. Using the technical options that were available in the respective time periods, humans have selected and generated populations of animals matching particular needs and purposes suited to specific climate and production systems. The result is the abundance of great phenotypic and genetic variation now found in domesticated animals including, for example, the more than 3000 cattle and 1300 pig breeds.

Table 1.1 Important milestones in the evolution of livestock breeding and animal biotechnology

~300.000 BC	The first humans emerge in East Africa
~12.000–15.000 BC	Begin of domestication of farm animals
~8000 BC	Separation of taurine and zebu cattle
Sixteenth century AD	Emergence of statistical concepts used for farm animal breeding
1677	Discovery of sperm cells
1780	First successful insemination (dog)
1866	First publication of Mendel's laws
1891	First successful ET (rabbit)
1934	First successful ET in sheep
>1940	Emergence of quantitative genetic concepts to accelerate genetic gain in livestock
1949	First successful ET in goat
>1970	Widespread field application of AI in farm animals
1971	First successful freezing/thawing of mammalian embryos (mouse)
1973	First successful freezing of bovine embryos
1980	First successful production of monozygotic twins by embryo splitting (sheep)
1982	First calf after transfer of in vitro produced embryos
1985	First transgenic farm animals (rabbits, sheep, and pigs) via microinjection
1985	First successful vitrification of mouse embryos
1985	First successful IVF in pig
1986	First successful embryonic cloning in sheep
1989	Birth of the first offspring (rabbits) after use of sex-sorted semen
>1990	Increasing use of QTLs in farm animals
1996	First successful cloning with somatic cells ("Dolly")
1998	First transgenic animal (sheep) after use of SCNT with transfected donor cells ("Polly")
>2000	Growing importance of MAS concepts
2001	Concept of genomic breeding value published; Publication of the human genome
2004	Chicken genome published
2006	Genome of dog and bee published
2009	Genome of domestic cattle and horse published
>2010	Growing implementation of GBV in important cattle breeds
2011	First pigs with a biallelic knockdown induced by the use of gene editing (ZFNs)
2012	Pig genome published
2013	First genetically modified pigs after use of CRISPR/Cas
2014	Sheep genome published
2017	Goat genome published

Abbreviations: *ET* embryo transfer, *AI* artificial insemination, *IVF* in vitro fertilization, *QTL* quantitative trait loci, *MAS* marker-assisted breeding, *SCNT* somatic cell nuclear transfer, *GBV* genomic breeding value, *ZFNs* zinc finger nucleases, *CRISPR/Cas* clustered regularly interspaced short palindromic repeats, *BC* Before Christ

This rich diversity of phenotypes has been a major attractor for evolutionary biologists and geneticists, including Charles Darwin who used the limited data then available as a key component in his theory of evolutionary biology in 1859 (Wang et al. 2014). Their endeavors, together with the wealth of new information stemming from the recent developments that allow detailed, cost-effective studies of individual animal genomes, have led to the accumulation of massive and complex datasets (Gerbault et al. 2014), requiring new modeling approaches to be developed that incorporate the latest statistical, population, and molecular genetics methodologies. The result of the interrogation of the data is an increasingly detailed understanding of domestication processes for all the major livestock species (Gerbault et al. 2014).

A major qualitative step in the evolution of systematic livestock breeding was made in the late nineteenth century with the introduction of statistical methodologies to systemic breeding practices. The initial application of statistical methods to animal breeding and genetics is mainly credited to Francis Galton (1822–1911) and Karl Pearson (1857–1936) who both worked before Mendel's law was rediscovered. One of their key findings was that on average, descendants from tall parents were smaller than their parents, while progeny from shorter parents was taller. This statistical regression of offspring on parent formed the basis of the more general heritability concept (Gianola and Rosa 2015). Subsequent development and application of this and other statistical concepts was critical for the scientifically based animal selection programs emerging in the twentieth century (Rothschild and Plastow 2014). Most animal breeding issues have a quantitative dimension that can be addressed via the application of one or more of the plethora of powerful statistical methodologies developed during the last four to five decades (Gianola and Rosa 2015). Application of these methodologies has allowed the recognition, introduction, and guided expansion of specific production traits to occur at an unprecedented rate. The emerging challenge for the livestock industry is to realize the potential of these advances to specific animal selection programs while maintaining sufficient genetic diversity for future innovations (Groeneveld et al. 2010).

1.2.2 Evolution of Scientifically Based Breeding Concepts

The twin foundations of the science-based breeding programs used in all modern livestock industries are quantitative genetics and reproductive biotechnology. From early on, there were two approaches to applying genetics to animal breeding (Blasco and Toro 2014). The first approach started with the rediscovery of Mendel's law and sought to identify inheritable chemical or molecular markers that could be used in genetic studies. Initial success came from the discovery of enzymatic polymorphisms, through the introduction of electrophoretic technologies in the 1960s that could be related to blood groups and/or coat color. While these studies revealed the potential of using the approach to following genetic variability among animals, it, disappointingly, only led to the identification of a few genetic variants that could be used to guide breeding strategies. The second approach can be traced back to Francis

Galton (1822–1911), a Victorian scientific polymath, who used a statistical approach in his studies of the expression of phenotypes among related animals. Both approaches aimed at promotion of genetic change in economically important productive traits (Blasco and Toro 2014) and subsequently became increasingly intermingled and eventually converged to exploit the genomic maps made available with improved DNA sequencing methods.

The genetic value of an animal is commonly described by its breeding value reflecting the major heritable traits being targeted for improvement in a specific breeding program. Developments in statistics and genomics have led to increasingly more accurate breeding values, thereby improving the rate of gain. In dairy cattle, selection was initially targeted at important milk parameters, such as milk yield, milk protein, and fat contents, other physiological factors being of minor importance or even neglected. Today breeding values recognize the importance of maintaining robust health in the herd or flock and include heritable physiological factors, such as longevity, claw, and udder health with the relative weighting for milk parameters significantly reduced. These breeding values are now recognized globally, thus facilitating the global exchange of valuable genetics.

1.2.3 Advent of DNA-Based Breeding Concepts

The rapid implementation of selection strategies based on DNA analysis became possible through what can only be described as truly impressive advances in DNA-analytical technology achieved since the initial attempts in the late 1960s, made with the simple tools then available (Shendure et al. 2017). Remarkable advances in multiple technologies have been made since that time, particularly in the last two decades. Procedures used to laboriously sequence a few kilo bases of DNA have now evolved to a stage where DNA studies commonly interrogate information derived from massive parallel sequencing of millions and myriads of DNA stretches. Significantly, this advance has been accompanied by a progressive and dramatic reduction in DNA sequencing costs to a point where being able to sequence whole genomes of individual humans and animals for a few hundred € or $US or even less (Shendure et al. 2017). A major driver for these developments have been human health issues, and the challenge of development and application of this capacity together with the growing recognition of the potential of the technology to individualizing medical treatment has, not unexpectedly, resulted in a rapidly expanding medical biotechnology industry. The livestock industry can expect similar major developments following the recent availability of sequences for all the major livestock genomes, including cattle, pigs, sheep, horses, poultry, goat, dogs, and cats (see chapter of Blasco and Pena in Volume II of this book). The first nearly complete draft of the human genome sequence was published in 2001, the outcome of >10 years of intensive work, involving many laboratories and a massive expenditure of money (Venter et al. 2001). The pace of development of cost-effective, reliable, and rapid sequencing procedures since that time is a major factor in establishing and

Table 1.2 Size of genomes of farm animals

	Number of chromosomes	Size of the genome (Gb)	Number of coding genes
Cattle	60	2,86	~22.000
Pig	38	2,76	~22.000–24.000
Sheep	54	2,71	~21.000
Poultry	34	1,2	~18.400
Horse	64	2,4–2,7	~20.000

1 Gb = 10^{-9} bp

refining the ever-growing library of complete animal genome sequences that now includes all major livestock species.

Detailed analysis of this valuable database has shown that animal genomes share a number of important features, most notably the finding that the total number of protein coding genes is only ~21.000–23.000 and that only a small proportion of it, usually 4–6% of the genome, is actively transcribed into proteins, the remaining major part of the genome being made up by repetitive sequences and epigenetic and retroviral elements, presumed, until very recently, to be uninvolved in the regulation of coding genes (Table 1.2). This viewpoint is being increasingly challenged by the finding that gene expression of an individual is being continually altered without any change in the genome's sequence. Recent research has identified some of these now called epigenetic processes, including methylation of DNA, alterations in the histone molecules that hold together DNA superstructures via methylation or acetylation or other biochemical modifications, and various RNA and Dicer protein-dependent processes that inhibit gene expression. In combination, the sum total of all these epigenetic marks in an individual is known as the epigenome.

Clearly, in the light of a growing appreciation of epigenomics and other unanticipated gene regulatory phenomena, our understanding of the significance of these noncoding elements needs analysis and revision. This is currently being undertaken through international collaboration, most notably through a project called ENCODE (Encyclopedia of DNA elements) (Kellis et al. 2014). Future refinement of breeding concepts will be increasingly dependent on the outputs of initiatives such as ENCODE to fully understand gene regulation and the role of both coding and non-coding DNA sequences in the expression of individual traits and their propagation in a given population. This is important to cope with anticipated and the unexpected challenges to future breeding enterprises. Developments in this field are of particular interest to livestock breeders as it is known that the lifetime health and productivity of animals derived by some reproductive technologies may be associated with alterations of the epigenome.

A major advance in the application of DNA analysis to animal breeding was made with the identification and introduction of QTLs (quantitative trait loci). Implemented in the mid-1990s in the dairy industry, it has since led to the discovery of a number of important QTLs in the various farm animal species. An important finding from use of QTLs was the identification of causal mutations for specific traits (Blasco and Toro 2014). The QTL strategy was succeeded by the concept of marker-assisted selection (MAS). This is essentially a three-step process that

includes the detection of several QTLs, followed by identification of the gene which causes the respective mutation and finally the increase of the frequency of the favorable allele by selection or by introgression (Blasco and Toro 2014). Early and prominent examples of the use of MAS are the halothane gene in pigs and the Booroola gene in sheep (Dekkers 2004).

MAS systems have now evolved further to what is called genomic selection (Meuwissen et al. 2001). This system was made possible through both identification of a dense set of informative markers that are, ideally, more or less evenly distributed across the genome and on cost-effective genotyping procedures. Genomic selection requires large testing populations and accurate phenotypic characterization (Meuwissen et al. 2001). The insights into gene sequences and their location on the chromosomes revealed through the broad-scale use of genomic selection ensure a constantly improving understanding of the genetic architecture of farm animals and many opportunities for the identification of the molecular identifiers of economically important traits.

The major technological advance already accelerating genomic projects in the major domestic species are chip arrays with several hundred thousand SNPs (single-nucleotide polymorphisms). Chips now available commercially target 750.000 SNPs for cattle, 56.000 SNPs for sheep, and 60.000 SNPs for pigs (Blasco and Toro 2014). Genomic selection by this means has a number of significant advantages over previous programs, most significantly when used to predict the breeding value in the born calves and even in early embryos. Already embryo analysis by this means has been shown to have greater accuracy in predicting breeding value than the classical pedigree index, with the additional benefit of it avoiding the costs and time-consuming maintenance of waiting bulls. Uptake of this approach to livestock selection by the cattle industry is well advanced, and the genomic breeding value (GBV) is increasingly being implemented into the breeding programs of major dairy and dual-purpose breeds, such as Holstein-Friesian and Simmental.

1.3 Evolution of Reproductive Biotechnology

1.3.1 History of Artificial Insemination (AI)

Artificial insemination (AI) was the first and remains the most widely used of the growing armory of reproductive technologies available to the livestock breeder. As a consequence, there is already a library of comprehensive reviews of the origins and history of AI and its impact on the animal breeding enterprises (e.g., Foote 1996; Vishwanath 2003; Ombelet and van Robays 2015; Orland 2017). Only the key advances in this still evolving technology are thus summarized below; for more detailed and informative accounts and references, see the reviews cited above.

The significance of semen in reproduction has been appreciated by most if not all cultures, since the earliest of times, with stories of attempt at AI part of the mythology of several cultures. It is generally accepted that the scientifically based AI traces back to the seventeenth century when development of the compound microscope

allowed the discovery and description of mammalian sperm cells from humans and dogs by Antoni van Leeuwenhoek and his assistant Johannes Hamm in 1678 in the Netherlands (Ombelet and van Robays 2015; Orland 2017). However, it was more than 100 years before the first documented success with AI was recorded: in the 1780ties in the human by the eminent scientist surgeon John Hunter, and by Lazzaro Spallanzani, an Italian physiologist, in a dog. Full appreciation of the potential value of AI to animal breeding only became evident in the late nineteenth century when it was made a specific subject of research (Orland 2017). Interestingly, it was Spallanzani, who also made the observation that human sperm became immotile when it accidently came in contact with snow, a seminal observation foreshadowing the use, 200 years later, of cryopreservation to store both sperm and ovum.

A major stimulus to this renewed interest in AI was the report in 1897 by Walter Heape, a British zoologist and embryologist based in Cambridge, of success in AI with rabbits, dogs, and horses. Significantly, his success laid the foundation, in 1932, of the Animal Research Station in Huntington Road in Cambridge, a facility that was to play a lead role in the development of not only AI but many of the other key reproductive technologies now in wide-scale use (Polge 2007). Important milestones in the subsequent history of AI include the development of dilution media to extend the use of single ejaculates and allow long-term storage through cryopreservation of sperm, the addition of antibiotics to semen samples to control bacterial contamination, and the development of freezing and cooling protocols compatible with high survival rates of sperm cells (Table 1.3).

The rate of adoption of AI by animal breeders varied from country to country, impeded in part by religious, moral, and social concerns about interference with the natural order of things. Russia led the way following the pioneering work by Ivanovich Ivanov, a biologist who, by 1907, had extended the use of AI to sheep and a range of other domesticated animals, including foxes and poultry. Japan and Denmark were also early AI adopters and innovators with Edward Sorensen together with Gylling Holm establishing the first cooperative AI-based breeding program in

Table 1.3 Important milestones in the history of artificial insemination (AI)

Year	Discoverer	Main finding
1677	Antoni von Leeuwenhoek	First picture of sperm cells
1780	Lazzaro Spallanzani	First insemination (in a dog)
1790	John Hunter	First vaginal insemination in human
1900	Ilya Ivanov	Development of semen extenders
1939	Gregory Pincus	First conception (rabbit) by AI
1949	Christopher Polge et al.	Discovery of cryoprotective functions of glycerol
1950	Robert Foote and R. Bratton	Addition of antibiotics to semen extenders
1953	Jerome Shumann	First pregnancy after AI with frozen sperm (human)
1978	Robert Edwards and P. Steptoe	First IVF baby (Baby Louise)
Since 1970s		Broad application of AI in farm animals, mostly cattle and pigs

Modified from Ombelet and van Robays (2015)

dairy herds in Denmark in 1936. The clear success of this program proved to be the stimulus needed to encourage the introduction and broad-scale uptake of AI in the USA and throughout the western world (Foote 1996; Vishwanath 2003). The stimulation of demand for animal products triggered by World War II and its aftermath dramatically increased the use of AI, particularly with dairy cows, where it was applied not only to improve the genetics of a given herd but also to gain control over Brucellosis and other prevailing venereal diseases. The accompanying investment in research led to a continuing series of important innovations that have evolved to the plethora of breeding technology options available today. Significant developments in AI resulting from this investment include not only reliable and robust technology for the collection, storage, and insemination of semen but equally importantly accompanying refinements in animal husbandry allowing estrus detection and regulation and standardized measures of fertility assessment. As a consequence, AI remains the primary method of choice for animal breeders around the globe seeking to improve the genetic quality of their stock through the realization of the genetic potential of valuable sires within a given population (Vishwanath 2003). For general breeding purposes, on average, 200–300 insemination doses can now be produced from a single bull ejaculate and stored frozen indefinitely; for a boar ejaculate, usually 10–20 insemination doses can be produced with semen freezing possible, but still at low efficiency and in small ruminants, one ejaculate can be extended to serve 10–30 ewes and successfully cryopreserved.

Today, AI is employed in more than 90% of all sexually mature female dairy cattle in countries with well-advanced breeding programs. The use of AI is also increasing in pig production enterprises with now more than 50–60% of sows served by AI on a global scale. The adoption of AI for use with low unit cost animals such as sheep and goats is less widespread but is still employed in the breeding of greater than 3.3 million sheep and 0.5 million goats annually with further growth anticipated following major refinements in estrus synchronization and insemination techniques and the need for flexibility in genotype of flocks to match fluxes in market demand for meat and fiber. AI is also now widely practiced in the poultry industry with the extremes of genotype found in extensively modified species such as the turkey making it obligatory. The clear benefits of AI have been such that robust and reliable AI procedures are now being available for most domesticated non-livestock species and increasingly for individual breeds of wild animals as a primary means of preserving threatened genotypes (Comizzoli et al. 2000; Comizzoli and Holt 2014).

It is long known that the sperm determines the sex of the potential offspring: when a Y-chromosome-bearing sperm fertilizes the oocyte, the resulting XY-constellation leads to a male phenotype; the XX chromosome set results in a female phenotype. In ancient time, the Greek philosopher Democritus from Abdera (~450 BC) suggested that the right testis produced only males, whereas the left testis produced only females. Subsequently, the lack of understanding of the basic biological principle mentioned above has prompted numerous methodological approaches to be tested in their ability to achieve separation of X- and Y-chromosome-bearing sperm. However, only the recent application of advanced flow cytometric systems, based on the small differences in DNA contents (3–6% depending on

species, with the Y-chromosome being smaller than the X-chromosome) between X- and Y-chromosome-bearing sperm, allows effective and reliable separation of living X- and Y-chromosome-bearing sperm for AI. A major breakthrough was reported in 1989, when fertilization with sex-separated semen was achieved with surgical insemination in the rabbit and several pups were born showing the desired sex (Johnson et al. 1989). Later improvements of sexing protocols provided sex-sorted semen in large enough quantities for use in bovine IVF (Cran et al. 1993). Nowadays, flow cytometry has been advanced to a stage that frozen/thawed sexed semen can be routinely supplied for bovine AI (Garner and Seidel Jr 2008) and is now being offered commercially by different companies around the globe. Thus the use of sexed semen in AI has rapidly emerged as an important new tool to enhance efficiency of dairy production.

1.3.2 History of Animal Embryo Transfer

A detailed history of embryo transfer (ET) can be found in the excellent publication from Betteridge (2003). Efforts to establish embryo transfer technology were made as early as the nineteenth century with a Canadian-English evolutionary biologist, George John Romanes (1848–1894), credited with the first, albeit unsuccessful, attempts. The first transfer of embryos resulting in live born offspring was achieved in rabbit by Walter Heape in 1890. Interestingly, Heape did his experiments at his home in Prestwich, near Manchester, using the rabbit breeds Angora and Belgian hare as embryo donors and recipients. This small-scale project typifies work in the biological sciences being carried out at the time. However, technological advances achieved in this way could still attract worldwide recognition through the intense network of interconnections established between biological scientists in the UK and elsewhere in the scientific world via the Royal Society and similar national and regional scientific bodies. This network was a major contributor to the rapid growth of understanding of reproductive biology that was to allow the full extension of ET to agricultural animals.

The late 1920s and early 1930s saw the beginnings of specific investment in developing ET for use in agriculture on both sides of the Atlantic. For example, the work of a group at the Institut für Allgemeine und Experimentelle Pathologie in Vienna, led by Artur Biedl, achieved a successful pregnancy in rabbits after 70 transfers in 1922 (Biedl et al. 1922). However, two centers in particular are identified with the next key advances in ET, one in Cambridge, Massachusetts, USA, and the other in Cambridge, UK. Cambridge, USA, was the site of one groundbreaking development in embryo transfer technology in 1936 by Gregory Pincus, an outstanding American endocrinologist and scientist. Six years previously he had reported a series of 21 embryo transfers in the rabbit that yielded 3 litters (Pincus 1930), an achievement stemming from his introduction of the use of anesthesia to allow direct exposure of and access to the oviducts and ovaries and a special pipette he had built to facilitate ET. However, the vast majority of these and his subsequent experiments suffered from the lack of knowledge of the need for synchrony between

the embryo and the recipient uterus, an appreciation he only made in 1936 when he and his coworker Kirsch recovered blastocysts that had developed following transfer of one- and two-cell embryos to the oviducts of rabbits at estrus, that is, before functional corpora lutea have been established (Pincus and Kirsch 1936). The recognition of the need for synchrony between donor and recipient provided the key to the development of robust and reliable ET for use in livestock breeding programs.

The first steps toward use of ET in livestock breeding had already been made in 1931 by Hartman and his colleagues at the Carnegie Laboratory of Embryology in Baltimore, USA, who harvested bovine two-cell embryos for the first time (Hartman et al. 1931; Miller et al. 1931). This was followed a year later by the first recorded actual transfer of livestock embryos by the group of Berry and Warwick at the Agricultural and Mechanical College in Texas, USA, who used ET to investigate causes of early embryonic loss in sheep and goats (Warwick et al. 1934; Warwick and Berry 1949). To honor this achievement, Dr. Berry became the first recipient of the Pioneer Award of the International Embryo Transfer Society (IETS) in 1982. World War II interrupted progress and development of ET techniques in Europe, but the prevalent food shortage from the war and its aftereffects urged research aimed at improving livestock breeding technologies including ET. In the UK, embryo transfer was identified as critical for the production of high-quality meat from beef cattle produced from dairy herds. This need was an important prompt for the Agricultural Research Council (ARC) Unit of Animal Reproduction at the Huntingdon Road in Cambridge, UK, the remarkable body of work on ET contributed by the Unit from then until its closure in 1986, making it a must go to scientific center in assisted reproductive technologies (ARTs). Among its early achievements were major advances in superovulation and the introduction by Lionel Edward Aston (Tim) Rowson, of nonsurgical collection of embryos in cattle breeds through his development of a catheter for transcervical recovery. As a consequence of the broad-spread interest triggered by these and subsequent developments in ET among breeders of both livestock, specifically cattle, robust and reliable ET protocols are now available for a large number of species (Table 1.4).

Important contributions to embryo transfer technology in other livestock species, such as sheep and pigs, came from the former Soviet Union (USSR) and Poland (Lopyrin et al. 1950, 1951; Kvasnitski 1951). An English translation of the Kvasnitski paper can be found in the proceedings of the conference held in May 2000 in Kiev, now Ukraine, that commemorated the 50th anniversary of the first successful porcine embryo transfer (Kvasnitski 2001).

From the 1970s onward, ET technology developed at a rapid pace through the work at the ARC Unit and other groups operative throughout the world. Important steps in this included the development of robust and reliable superovulation and synchronization protocols based on the better understanding of reproductive endocrinology and physiology, the use of frozen semen, and the implementation of nonsurgical transfer and collection techniques. Important advances were also made in the development of media suitable for the holding and culture of early embryos. Field application of the new technologies was advanced in 1972, through an instruction course on ET technology organized in Cambridge, UK, which brought together

Table 1.4 First successful (with the delivery of live offspring) embryo transfers in different species

Year	Author	Country	Species
1891	Heape	UK	Rabbit
1933	Nicholas	USA	Rat
1934	Warwick et al.	USA	Sheep
1942	Fekete and Little	USA	Mouse
1949	Warwick and Berry	USA	Goat
1951	Willett et al.	USA	Cattle
1951	Kvasnitski	UdSSR (Ukraine)	Pig
*1964	Mutter et al.	USA	Cattle
1968	Chang	USA	Ferret
1974	Oguri and Tsutsui	Japan	Horse
1976	Kraemer et al.	USA	Primate
1978	Steptoe and Edwards	UK	Human
1978	Shriver and Kraemer	USA	Cat
1979	Kinney et al.	USA	Dog

*Transcervical transfer

a group of veterinarians and scientists from around the globe. This group later played a crucial role in forming the International Embryo Transfer Society (IETS) (Carmichael 1980; Schultz 1980), now regarded as the lead scientific forum for the exchange of new ideas on embryo transfer and related technologies. In 2016, the name of the society was changed to "International Embryo Technology Society," to better reflect the importance of the emerging embryo-related techniques such as in vitro fertilization, freezing, or cloning.

Another important step toward practical ET techniques was the report of the first successful freezing of a mammalian embryo, the mouse (Whittingham 1971), an advance based on the demonstration by M.C. Chang, in 1947, of the feasibility of this by his successful transfer of rabbit embryos that had been cooled to 10 °C (Chang 1947). The report of the first successfully frozen/thawed bovine embryos quickly followed (Wilmut and Rowson 1973). This success allowed animal breeders not only to freeze and store valuable gene stock for transfer to appropriate recipients as needed but opened up the way for global exchange of gene stock through frozen embryos as well as sperm. Refinements in freezing protocols have been rapid, due in part to the co-interest in cryopreservation of human tissues. This had led to a number of different freezing protocols now being available for freezing bovine and other livestock embryos. The number of transfers of bovine embryos, both freshly collected and frozen/thawed, increased significantly in the last decade from ~823.200 in 2006 (Thibier 2008) to up to ~965.000 embryos in 2016 (Perry 2017). While ET is widely used in dairy and parts of beef cattle, it is much less applied in pigs (few thousand ETs), small ruminants (few hundred ETS), and horses (few thousand ETs) (Perry 2017). Thus, embryo transfer technology is now an integral part of modern breeding concepts for cattle and is widely applied across the globe. However, while embryo transfer technology allows a better exploitation of

the genetic potential of the female germ pool than AI, it is still only used in the top 1–2% of a breeding population.

A major expansion of interest in ET technology followed the landmark achievement in human reproductive medicine with the birth of Louise Brown in 1978 in Oldham, UK, following in vitro fertilization and transfer procedures developed by Robert Edwards, a Cambridge, UK, physiologist, and Patrick Steptoe, a surgeon from Oldham, UK (Edwards and Steptoe 1978). The foundation stones for Edwards' success were laid nearly 20 years earlier in what has been described as a golden age in IVF studies (Bavister 2002). Highlights of this era were the reports of Anne McLaren and John Biggers of successful development and birth of mice cultivated in vitro as early embryos (McLaren and Biggers 1958) and, a year later, MC Chang's findings that in vitro fertilized rabbit eggs could develop normally following transfer to surrogate mothers (Chang 1959).

A prime motivation for Edwards' in vitro fertilization was his interest in addressing the high incidence of infertility in humans, in particular the growing number of women in the post-pill era with infertility due to hydrosalpinx, a blockage in their fallopian tubes that could be traced to a prior reproductive tract infection, most commonly chlamydia. Demonstrating that in vitro fertilization of human oocytes was possible was the first step (Edwards et al. 1969); the next was for Steptoe to use his skills in laparoscopy to develop minimally invasive procedures allowing repeated collection of oocytes that Edwards could fertilize in vitro and reimplant in the uterus thus by-passing the damaged tubes and achieving pregnancy. Their epoch-making achievement was the culminating point of Robert Edwards lifetime of pioneering research in human infertility and earned him the Nobel Prize in 2010 (Johnson 2011).

IVF is now used to address a wide range of fertility issues, and the number of babies born from assisted reproductive technologies (ART) is increasing rapidly: their numbers have more than quadrupled since 1995, and to date, >5 million babies worldwide have been born after ART (ESHRE 2009). ART births constitute 1.5–4.5% of all births in the USA and other countries such as the UK (Sunderam et al. 2018; HFEA 2011). In livestock breeding, the technology initially lagged behind that in human, with the first successful IVF from in vivo matured oocytes in cattle in 1982 (Brackett et al. 1982) and entirely from IVM/IVF/IVC in 1987 (Fukuda et al. 1990) and in the pig in 1985 (Cheng et al. 1986). IVM/IV + IVC have now been refined to a stage that it is possible to repeatedly harvest oocytes by laparoscopic and nonsurgical techniques, mature and fertilizing the harvested oocytes in vitro, followed by culture of the resultant zygotes to the blastocyst stage for transfer to synchronized recipients. These IVM/IVF/IVC procedures are now being widely used for experimental studies and commercially as well, for recovery of valuable gene stock postmortem (usually from abattoirs), and to reduce the generation interval via collection of oocytes from juvenile animals (JIVET). Current global figures revealed a total of ~450.000 entirely in vitro produced bovine embryos that had been transferred to recipients with geographical emphasis in South America (Perry 2017). The application of IVM/IVF/IVC combined with cryopreservation

procedures in the introduction of highly productive genotypes is now supporting development of China's dairy herds.

1.3.3 "Dolly" and Beyond

The birth of an ewe named "Dolly" in Scotland in July 1996 opened up a new world of possibilities for animal breeders. Dolly's distinction was the fact that she was the first cloned mammal derived from a fully differentiated adult cell (Wilmut et al. 1997), a fact that challenged the then ruling paradigm that genes not required in the development of specific tissues were lost or permanently inactivated (Weissmann 1893). From the animal breeders' perspective, this was interpreted as limiting any developments in cloning technology to cells from early embryos, that is, before cells become committed to their specific differentiation pathway. This limiting paradigm was a consequence of studies made with amphibian embryos in 1952 by Robert Briggs and Thomas J. King in Philadelphia, USA. Using the amphibian species *Rana pipiens*, and the nuclear transfer procedures they had specifically developed for the purpose, they showed that while normal tadpoles could be obtained after transplanting the nucleus of a blastula cell into the enucleated egg, tadpole development became increasingly restricted as cells underwent differentiation (Briggs and King 1952). This led to the hypothesis that the closer the nuclear donor is developmentally to early embryonic stages, the more successful nuclear transfer is likely to be. Support for this viewpoint came from John Gurdon, an Oxford, UK, based developmental biologist, who used another amphibian, the frog *Xenopus laevis*, as model species. *Xenopus* has some distinct advantages over *Rana pipiens*, because (1) the embryos can be grown to sexual maturity in less than a year, (2) *Rana pipiens* lives more than 4 years, and (3) *Xenopus* frogs can be induced to lay eggs throughout the year after hormonal injections. In contrast, *Rana pipiens* and other frogs are strictly seasonal. Gurdon showed that only with less differentiated donor cells, he could achieve development and developmental rates dropped when more differentiated cells were used as donors (Gurdon 1960, 1962, 2017). This viewpoint prevailed for many years and had a strong influence on the design of experiments in the 1970s and 1980s.

Cloning of mammals became possible when laboratory equipment became available in the late 1960s and early 1970s that allowed micromanipulation of the much smaller mammalian eggs (100–130 μm in diameter, i.e., about one tenth of the diameter of the amphibian egg). The first report on cloning in mammals was by Illmensee and Hoppe (1981) who reported the birth of three cloned mice after transfer of nuclei from the inner cell mass cells of a blastocyst into enucleated zygotes. However, these results could not be repeated, with other researchers finding that development was arrested following the transfer of the nucleus of a zygote or two-cell embryos into an enucleated zygote (McGrath and Solter 1983). The same researchers also found no development when nuclei from donor cells from later development stages were used (McGrath and Solter 1984). This led the authors to

conclude that cloning of mammals by nuclear transfer would be biologically impossible, presumably due to the rapid loss of totipotency in developing embryonic cells. The challenge to this viewpoint came only few years later in 1986, from Steen Willadsen, a Danish developmental biologist working in the ARC Unit in Cambridge, UK, through his demonstration that nuclei obtained from blastomeres from cleavage stage ovine embryos could be inserted into enucleated oocytes and viable lambs obtained following transfer to recipient ewes (Willadsen 1986). This major technical advance, together with the later finding that donor cells could even be obtained from the inner cell mass (ICM) of bovine blastocysts (Sims and First 1994), established a base for the following successful embryonic cloning of rabbits, mice, pigs, cows, and monkeys (for review see Niemann et al. 2011).

The possibility of cloning mammals through somatic cells was heralded in 1996/1997 through the publication of two landmark papers by the group at the Roslin Institute, Edinburgh, Scotland, UK. Their initial achievement was the demonstration of the feasibility of deriving donor nuclei from an established cell line derived from a day 13 ovine conceptus and maintained in vitro for several passages (Campbell et al. 1996). This remarkable success they attributed to their synchronizing of the cell cycle of the donor cells through lowering the concentrations of serum in the culture medium, thus causing the cells to exit the cell cycle and hold at the Go stage. Transfer of donor cells from these quiescent cell lines to enucleated matured oocytes and transfer of the reconstructed embryos into synchronized recipient ewes resulted in the birth of two healthy cloned lambs called "Megan" and "Morag." Their achievement encouraged the group to extend their studies to somatic cells derived from mammary epithelial tissue that led to the birth of "Dolly" the following year (Wilmut et al. 1997). The prospect of translation of these findings into animal breeding enterprises was enhanced by "Dolly" living a rather normal life at the Roslin Institute until she had to be euthanized in February 2003 due to a fatal pulmonary disease caused by the adenomatosis virus endemic in Scottish sheep flocks. The significance of this advance is documented through the exhibition of Dolly's preserved remains in the Science and Technology Galleries of the National Museum of Scotland, Edinburgh (Fig. 1.1). Interestingly, Dolly is one of the museum's most popular exhibits and has become a symbol of Scottish national pride (García-Sancho 2015). Important steps into the evolution of somatic cloning are depicted in Table 1.5.

Dolly's birth launched a heated ethical debate worldwide and sparked a series of science fiction stories. Initially, the origin of Dolly from a fully differentiated donor cell was questioned by many scientists. However, in the next 5–10 years, the validity of their claims was proven and the feasibility of somatic cell cloning fully realized and established as an important tool in research. Somatic cloning by somatic cell nuclear transfer (SCNT), resulting in the production of live clones, has now been successfully extended to more than two dozen species, including sheep, cattle, mouse, goat, pig, cat, rabbit, horse, rat, dog, ferret, red deer, buffalo, gray wolf, camel, and very recently nonhuman primates (see Niemann 2016; Liu et al. 2018), and, despite a slow start, has been developed to a stage where it is now being offered commercially in all the important agricultural species, including cattle, pigs, and horses.

The underlying mechanisms that determine success in somatic nuclear transfer are still a subject of active research. One initial hypothesis was that the clones only

Fig. 1.1 Dolly, the first mammal cloned from a somatic cell, can be visited in the Scottish National Museum in Edinburgh

arose from a subpopulation of stem cells (Hochedlinger and Jaenisch 2002). However, this was short lived as evidence built up showing that differentiated somatic cells can successfully be employed in SCNT. The reprogramming of the genome following nuclear transfer causes dramatic changes of the epigenetic landscape of the donor cell consistent with the expression profile of the differentiated cells being abolished and a new, embryo-specific expression profile established to drive embryonic and fetal development (Niemann et al. 2008). It is now known that such epigenetic reprogramming involves the erasure of the gene expression program of the respective donor cell and the reestablishment of the well-orchestrated sequence of expression of the estimated 10,000–12,000 genes critical for early embryonic development. Through Dolly, mammalian development is now established as having high plasticity with significant implications for many areas in the natural sciences and in public debate.

Soon after "Dolly" the sheep was born, the journal "Cloning" was launched in 1999 to cover the emerging new information in this area. The journal was expanded in 2002 and 2010 to include all mechanisms of cellular reprogramming and is now called "Cellular Reprogramming" (Wilmut and Taylor 2018). This reflects the dramatic impact of somatic cell cloning not only on animal breeding but in both the biological and medical sciences. One use is in the derivation of so-called induced

Table 1.5 Important milestones in the development of somatic cloning via somatic cell nuclear transfer (SCNT)

Author	Year	Main findings
Spemann	1938	Embryonic development and early differentiation
Briggs and King	1952	Viable tadpoles from nuclei transplanted from blastula stages in *Rana pipiens*; nuclei are multipotent
Gurdon	1962	Viable tadpoles from intestinal epithelial cells in *Xenopus laevis*; nuclei are multipotent
Gurdon and Uehlinger	1966	Fertile adult frogs from intestinal epithelial cells of feeding tadpoles in *Xenopus laevis*; nucleus is still totipotent
McGrath and Solter	1984	Arrested development of reconstructed mouse embryos; claim: Mammalian cloning is biologically impossible
Willadsen	1986	Successful nuclear transfer-based cloning using embryonic donor cells in sheep (8–16 cells)
Tsunoda et al.	1987	Successful nuclear transfer in mice using 4–8 cell embryos as donors
Prather et al.	1987	Successful cloning of cattle by using 2–32 cell stage embryos as donors
Sims and First	1994	Successful cloning of cattle by using cultured cells from the inner cell mass (ICM) of blastocysts
Campbell et al.	1996	Successful cloning of sheep by using 13-days-old cultured fetal donor cells
Wilmut et al.	1997	Dolly, the sheep, successful cloning from a fully differentiated (mammary epithelial) cell
Cibelli et al.	1998	Successful somatic cloning of cattle using fibroblasts as donors
Wakayama et al.	1998	Successful somatic cloning of mice using adult cells
Many different authors	Since 1998	>24 species have been successfully cloned up to 2018
Liu et al.	2018	Successful cloning of nonhuman primate

Modified from Gurdon (2017)

pluripotent stem cells (iPSCs) in 2006 (Takahashi and Yamanaka 2006), an advance which earned S. Yamanaka the Nobel Prize together with John Gurdon in 2012 and established iPSCs as important tools for derivation of patient-specific therapeutic stem cells and regenerative medicine. In the biological sciences, SCNT has proven to be a research tool of great value in the study of early development and epigenetic mechanisms governing the expression of genes that regulate embryonic and fetal development (Kues et al. 2008; Niemann 2014).

SCNT is now developed to a stage where it has commercial application in major farm animals, including cattle, pigs, and horses. However, its main impact on animal breeding will not be through cloning of existing genomes but through its use as a route allowing the full armory of genome editing tools to be applied to the animal genome, allowing precise modification of existing genes or precise insertion of new genes in the animal genome.

1.4 Genome Editing and Precision Breeding

The demonstration, in 1980, by Jon W. Gordon and Frank Ruddle at Yale University, USA, that it was possible to introduce new and functional genetic material into the germline of laboratory rodents heralded a new era in animal breeding. Called transgenesis, it was achieved by microinjection of foreign DNA into oocytes shortly after fertilization (Hammer et al. 1985). The potential application of this powerful new tool was immediately recognized, and within 5 years the creation of the first genetically modified farm animals, including rabbits, pigs, and sheep, had been achieved (Hammer et al. 1985).

However, the microinjection approach to germline modification proved to be highly inefficient in practice and had other major limitations due the fact that it only allowed additive gene transfer and that the introduced DNA was integrated randomly in the recipient genome and a frequent incidence of mosaicism. These limitations were only overcome with the development of cell-based gene transfer methods realized following the confirmation of the feasibility of using SCNT by the birth of Dolly. SCNT-based procedures were quickly developed that now allow the full application of DNA editing technology to be applied to the somatic cells in culture prior to the introduction of the modified genome into the germline. The introduction of SCNT and its capacity to allow the selection and use of highly defined donor cells dramatically improved the production of genetically modified livestock. As a consequence, a whole new range of useful application models became available not only for rodents and other species used in basic research but for various livestock species with new traits of interest to agricultural and biomedical enterprises (Laible et al. 2015). However, cell-mediated transgenesis was still hampered by the inability to produce animals with targeted genetic modifications. This was at least partly due to the fact that in farm animals, in contrast to laboratory species (mouse and rat), robust and reliable procedures for the establishment of true pluripotent stem cell cultures have not yet been achieved (Nowak-Imialek and Niemann 2012). Primary cells only have a limited lifespan in culture, and being limited to their use in SCNT was not compatible with the high selection needed for targeted mutations, thus severely limiting the extent of the genetic modification that could be achieved.

This situation changed dramatically with the introduction of genome editing technologies based on the use of DNA nucleases (see Petersen and Niemann 2015). These molecular scissors, including zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and the CRISPR/Cas (clustered regularly interspaced short palindromic repeats) system, allow precise modifications of the genome. In animals all three nucleases can be applied either via microinjection into early fertilized eggs (zygotes) or after transfection into donor cells that are subsequently used in somatic cloning. Within a few years following their introduction, numerous research groups have described the successful production of genetically modified cattle, pigs, and sheep covering a range of potentially useful genetic modifications, both for agricultural and biomedical application (Petersen and Niemann 2015; Telugu et al. 2017).

For the first time, it became possible to overcome the limitations of the glacially slow classical breeding and selection processes traditionally used in agricultural enterprises. Using the new technologies of genome editing, new phenotypes can be produced and introduced within a single generation (Laible et al. 2015). Furthermore, with the capacity to target and edit individual genes or noncoding sequences in the genome in combination with the use of homologous recombination protocols, to introduce new DNA sequences provides the basis for establishing a whole new world of opportunities for animal breeding enterprises.

To date, only a limited number of products from genetically modified animals have been approved for use through the national supervisory bodies established to monitor and govern the use of these technologies. All were derived by conventional transgenic technologies, including recombinant human antithrombin (ATryn^{R}) from goat milk for prophylactic treatment of hereditary antithrombin deficiency within a surgery, recombinant C1 esterase inhibitor from rabbit milk for treatment of hereditary angioedema (HAE) (Ruconest®), and Kanuma (sebelipase alfa®), a recombinant human enzyme that is produced in egg white of hens to treat lysosomal acid lipase deficiency. Pigs and other livestock with enhanced production traits have been developed, but only one has been accepted for commercial use, namely, the AquAdvantage Atlantic salmon from the company Aquabounty. Of concern is that the fish which grows twice the size of the normal Atlantic salmon over the same time period only received official approval from the FDA, the supervisory body in the USA, in 2015, 20 years after its development and after a major regulatory battle. It will be interesting to see how products from animals derived from gene editing will be legalized as similar genetic changes may occur naturally, making it difficult, if not impossible, to identify the origin of the mutation. The recent acceptance in March 2018 of the safety of products derived through gene editing in food plants by the FDA is encouraging. The genomic maps of both plants and farm animals are constantly being refined, and a wealth of new opportunities for genomic editing that majorly expand genetic diversity from a variety of important application perspectives can be confidently anticipated (Petersen and Niemann 2015; Telugu et al. 2017).

1.5 Future Perspectives

Modern animal breeding strategies, mainly based on population genetics, novel molecular tools, and assisted breeding technologies (ARTs) such as AI and ET, have significantly increased the performance of domestic animals. This forms the basis for a regular supply of high-quality animal-derived food and fiber at competitive prices. For example, in both Australia and the USA, Holstein-Friesian dairy bovine milk production increased annually by about 1%, corresponding to 40–80 kg/cow/year, between 1980 and 2010 (Hayes et al. 2013). Gains that played an important part in the reduction are seen in the costs of milk and milk products. Similar gains were achieved in the efficiency of production of other animal-derived food products, such as meat and eggs.

The introduction of precision breeding concepts based on genome editing is an important step in allowing the necessary developments required to address compounding challenges in global food security, environmental sustainability, and animal welfare (Rothschild and Plastow 2014). It is predicted that by the year 2050, the global population will have grown up to 9.5–10 billion people. This growth will take place mainly in developing countries and in major urban areas requiring a dramatic increase in food production, including animal-derived protein. Estimates of future need for meat products indicate that meat production will need to increase by at least 70% to cope with this future demand. As the majority of arable land is already in production, there is a clear challenge to livestock breeders to increase efficiency of food production from both intensive and non-intensive animal production enterprises in a sustainable manner (Telugu et al. 2016). Encouragingly, considerable genetic variation for traits contributing to efficiency improvements in all livestock species still exists (Hayes et al. 2013). Realizing the full potential of these traits and the introduction of new traits will require the precision breeding concepts introduced in this brief history. DNA-based breeding concepts and genome editing are critical for ensuring an efficient and sustainable future for both plant- and animal-based agricultural enterprises. Further development and acceptance of bioengineered products will also be of immense medical importance in the generation of models for human diseases, xenotransplantation, the production of pharmaceutically active proteins, environmental remediation, and regenerative medicine.

The USDA has recently accepted (March 2018) that with precision editing now possible mutations would be indistinguishable from rare but possible natural mutations and stated that it does not and has no plans to regulate gene editing of plants or crops but will still treat plants with introduced foreign genes as GMOs (genetically modified organisms). Experience gained from repeated attempts to gain acceptance of genetically modified meat products suggests that there is still a way to go for even the most subtle gene modifications. The pathway to public acceptance of genome editing technologies in farm animals is probably an indirect one through initial demonstrations of its safety and value in addressing issues of animal welfare, human health, and sustainability. Procedures from genomic editing in animals must be rigorously screened for off-target mutations to avoid any violation of the integrity of the animal. This is now entirely feasible using advanced CRISPR/Cas and similar systems. The value of persisting in seeking to introduce this approach more broadly in the livestock sector has been confirmed by the recent demonstration that genome editing can be used to increase the genetic gain in farm animal breeding in both the short- and medium-term perspective. By applying gene drive concepts using genome editing tools, increasing the allele frequency using gene drive mechanisms would accelerate genetic gain even further and without the risk of increased inbreeding (Gonen et al. 2017). This viewpoint is supported by a recent simulation study that revealed that this approach could be used to refine the increase in genetic gain through accelerating the increase in the frequency of favorable alleles and reducing the time to fix them in germlines; labeling nucleotides, for a more rapid targeting of quantitative traits; and finally increasing the efficiency of converting

genetic variation into genetic gain (Gonen et al. 2017), all desirable capacities for inclusion in future breeding concepts.

In summary, researchers and animal breeders now have tools in hand to modify the genome in a previously unprecedented very precise manner. The potential to rapidly increase favorable genes in a given population is an important step toward achieving genetic gain and modulating economically important gene loci. These opportunities need to be exhaustively explored and their potential fully assessed as they are of vital importance to development of the animal enterprises needed to combat the looming challenges to food security from the hyperbolically increasing demands and predicted climatic and environmental uncertainties. These advances need to be carried out in a manner that ensures sufficient transparency and information to the public and decision-makers so that there is a general understanding of the importance and need for full support for initiatives in this area.

References

Bavister BD (2002) Early history of *in vitro* fertilization. Reproduction 124:181–196

Beja-Pereira A, Caramelli D, Lallueza-Fox C et al (2006) The origin of European cattle: evidence from modern and ancient DNA. Proc Natl Acad Sci U S A 103:8113–8118

Betteridge KJ (2003) A history of farm animal embryo transfer and some associated techniques. Anim Reprod Sci 79:203–244

Biedl A, Peters H, Hofstätter R (1922) Experimentelle Studien über die Einnistung und Weiterentwicklung des Eies im Uterus. Z Geburtshilfe Gynäk 84:59–103

Blasco A, Toro MA (2014) A short critical history of the application of genomics to animal breeding. Livest Sci 166:4–9

Brackett BG, Bousquet D, Boice ML, Donawick W, Evans JF, Dressel MA (1982) Normal development following in vitro fertilization in the cow. Biol Reprod 27:147–158

Bradford MW, Bradley DG, Luikart G (2003) DNA markers reveal the complexity of livestock domestication. Nat Rev Genet 4:900–910

Briggs R, King TJ (1952) Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. Proc Natl Acad Sci U S A 38:455–463

Campbell KHS, McWhir J, Ritchie WA et al (1996) Sheep cloned by nuclear transfer from a cultured cell line. Nature 380:64–66

Carmichael RA (1980) History of international embryo transfer society I. Theriogenology 13:3–6

Chang MC (1947) Normal development of fertilized rabbit ova stored at low temperature for several days. Nature 159:602–603

Chang MC (1959) Fertilization of rabbit ova *in vitro*. Nature 179:466–467

Chang MC (1968) Reciprocal insemination and egg transfer between ferrets and mink. J Exp Zool 168:49–60

Cheng WTK, Polge C, Moor RM (1986) In vitro fertilization of pig and sheep oocytes. Theriogenology 25:146 (abstr)

Cibelli J, Stice SL, Golueke PJ et al (1998) Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280:1256–1258

Comizzoli P, Holt WV (2014) Recent advances and prospects in germplasm preservation of rare and endangered species. Adv Exp Med Biol 753:331–356

Comizzoli P, Mermillod P, Mauget R (2000) Reproductive biotechnologies for endangered mammalian species. Reprod Nutr Dev 40:493–504

Connolly J, Colledge S, Dobney K et al (2011) Meta-analysis of zooarchaeological data from SW Asia and SE Europe provides insight into the origins and spread of animal husbandry. J Archeol Sci 38:538–545

Cran DG, Johnson LA, Miller NGA, Cochrane D, Polge C (1993) Production of bovine calves following separation of X- and Y-chromosome bearing sperm and in vitro fertilization. Vet Rec 132:40–51
Dekkers J (2004) Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J Anim Sci 82(E.Suppl):E313–E328
Diamond J (2002) Evolution, consequences and future of plant and animal domestication. Nature 418:700–707
Edwards RG, Steptoe PC (1978) Birth after the reimplantation of a human embryo. Lancet 312:366
Edwards RG, Bavister BD, Steptoe PC (1969) Early stages of fertilization in vitro of human oocytes matured in vitro. Nature 221:632–635
European Society of Human Reproduction (ESHRE) (2009) ART fact sheet. In: Embryology ESHRE. European Society of Human Reproduction (ESHRE), 2009
Fekete E, Little CC (1942) Observations on the mammary tumor incidence of mice born from transferred ova. Cancer Res 2:525–530
Foote RH (1996) Review: dairy cattle reproductive physiology research and management – past progress and future prospects. J Dairy Sci 79:980–990
Foote RH, Bratton RW (1950) The fertility of bovine semen in extenders containing sulfanilamide, penicillin, streptomycin and polymyxin. J Dairy Sci 33(8):544–547
Fukuda Y, Ichikawa M, Naito K, Toyoda Y (1990) Birth of normal calves resulting from bovine oocytes matured, fertilized, and cultured with cumulus cells in vitro up to the blastocyst stage. Biol Reprod 42:114–119
García-Sancho M (2015) Animal breeding in the age of biotechnology: the investigative pathway behind the cloning of Dolly the sheep. HPLS 37:282–304
Garner DL, Seidel GE Jr (2008) History of commercializing sexed semen in cattle. Theriogenology 69:886–895
Gerbault P, Allaby RG, Boivin N et al (2014) Storytelling and story testing in domestication. Proc Natl Acad Sci U S A 111:6159–6164
Gianola D, Rosa GJM (2015) One hundred years of statistical developments in animal breeding. Annu Rev Anim Biosci 3:19–56
Gonen S, Jenko J, Gorjanc G et al (2017) Potential of gene drives with genome editing to increase genetic gain in livestock breeding programs. Genet Sel Evol 49:3. https://doi.org/10.1186/s12711-016-0280-3
Groenen MAM (2016) A decade of pig genome sequencing: a window on pig domestication and evolution. Genet Sel Evol 48:23. https://doi.org/10.1186/s12711-016-0204-2
Groeneveld LF, Lenstra JA, Eding H et al (2010) Genetic diversity in farm animals – a review. Anim Genet 41:6–31
Gurdon JB (1960) The developmental capacity of nuclei taken from differentiating endoderm cells of Xenopus laevis. J Embryol Exp Morphol 8:505–526
Gurdon JB (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J Embryol Exp Morphol 10:622–640
Gurdon JB (2017) Nuclear transplantation, the conservation of the genome, and prospects for cell replacement. FEBS J 284:211–217
Gurdon JB, Uehlinger V (1966) "Fertile" intestine nuclei. Nature 210:1240–1241
Hammer RE, Palmiter RD, Pursel VG et al (1985) Production of transgenic rabbits, sheep and pigs by microinjection. Nature 315:680–683
Hartman CG, Lewis WH, Miller FW et al (1931) First findings of tubal ova in the cow, together with notes on oestrus. Anat Rec 48:267–275
Hayes BJ, Lewin HA, Goddard ME (2013) The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. Trends Genet 29:206–214
Heape W (1891) Preliminary note on the transplantation and growth of mammalian ova within a uterine foster mother. Proc R Soc Lond Biol Sci 48:457–459
Heape W (1897) The artificial insemination of mammals and subsequent possible fertilization or impregnation of their ova. Proc R Soc Lond B 61:52–63

Hochedlinger K, Jaenisch R (2002) Monoclonal mice generated by nuclear transfer from mature B and T donor cells. Nature 415:1035–1038
Human Fertilisation and Embryology Authority (HFEA) (2011) Fertility treatment in 2011: trends and figures in UK: Human Fertilisation and Embryology Authority (HFEA)
Illmensee K, Hoppe PC (1981) Nuclear transplantation in Mus musculus: developmental potential of nuclei from preimplantation embryos. Cell 23:9–18
Johnson MH (2011) Robert Edwards: the path to IVF. Reprod Biomed Online 23:245–262
Johnson LA, Flook JP, Hawk HW (1989) Sex selection in rabbits: live births from X and Y sperm separated by DNA and cell sorting. Biol Reprod 41:199–203
Kellis M, Wold B, Snyder M (2014) Defining functional DNA elements in the human genome. Proc Natl Acad Sci U S A 17:6131–6138
Kinney GM, Pennycook JW, Schriver MD et al (1979) Surgical collection and transfer of canine embryos. Biol Reprod 20:96A
Kraemer DC, Moore GT, Kramen MA (1976) Baboon infant produced by embryo transfer. Science 192:1246–1247
Kues WA, Sudheer S, Herrmann D et al (2008) Genome-wide expression profiling reveals distinct clusters of transcriptional regulation during bovine preimplantation development in vivo. Proc Natl Acad Sci U S A 105:19768–19773
Kvasnitski AV (1951) Interbreed ova transplantations (in Russian). Soc Zooteckh 1:36–42 (Anim Breed Abstr 19:224)
Kvasnitski AV (2001) Research on interbreed ova transfer in pigs. Theriogenology 56:1285–1289
Laible G, Wei J, Wagner S (2015) Improving livestock for agriculture – technological progress from random transgenesis to precision genome editing heralds a new area. Biotechnol J 10:109–120
Larson G, Albarella U, Dobney K et al (2007) Ancient DNA, pig domestication, and the spread of the Neolithic into Europe. Proc Natl Acad Sci U S A 104:15276–15281
Liu Z, Cai Y, Wang Y (2018) Cloning of macaque monkeys by somatic cell nuclear transfer. Cell 172:881–887
Lopyrin AI, Loginova NV, Karpov PL (1950) Changes in the exterior of lambs as a result of interbreed embryonic transfer (in Russian). Dokl Adad Nouk SSSR Ser Biol 74:1019–1021 (Anim Breed Abstr 19, Abstr 1262)
Lopyrin AI, Loginova NV, Karpov PL (1951) The effect of changed conditions during embryogenesis on the growth and development of lambs (in Russian). Sov Zootheh 6:83–95 (Anim Breed Abstr 20, Abstr 729)
McGrath J, Solter D (1983) Nuclear transplantation in mouse embryos. J Exp Zool 228:355–362
McGrath J, Solter D (1984) Inability of mouse blastomere nuclei transferred to enucleated zygotes to support development in vitro. Science 226:1317–1319
McHugh DE, Bradley DG (2001) Livestock genetic origins: goats buck the trend. Proc Natl Acad Sci U S A 98:5382–5384
McLaren A, Biggers JD (1958) Successful development and birth of mice cultivated *in vitro* as early embryos. Nature 182:877–878
Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829
Miller FW, Swett WW, Hartman CG et al (1931) A study of ova from the Fallopian tubes of dairy cows, with a genital history of the cows. J Agric Res 43:627–636
Mutter LR, Graden AP, Olds D (1964) Successful non-surgical bovine embryo transfer. AI Digest 12:3
Nicholas JS (1933) Development of transplanted rat eggs. Proc Soc Exp Biol Med 30:1111–1113
Niemann H (2014) Epigenetics of cloned livestock embryos and offspring. In: Cibelli J, Gurdon J, Wilmut I, Jaenisch R, Lanza R, West MD, KHS C (eds) Principles of cloning. Elsevier, Amsterdam, pp 453–463
Niemann H (2016) Epigenetic reprogramming in mammalian species after SCNT-based cloning. Theriogenology 86:80–90

Niemann H, Tian XC, King WA et al (2008) Epigenetic reprogramming in embryonic and foetal development upon somatic cell nuclear transfer. Reproduction 135:151–163
Niemann H, Kues WA, Lucas-Hahn A et al (2011) Somatic cloning and epigenetic reprogramming in mammals. In: Atala A, Lanza R, Thomson JA, Nerem R (eds) Principles of regenerative medicine. Elsevier., ISBN: 978-0123814227, Amsterdam, pp 129–158
Nowak-Imialek M, Niemann H (2012) Pluripotent cells in farm animals: state of the art and future perspectives. Reprod Fertil Dev 25:103–128
Oguri N, Tsutsumi Y (1974) Non-surgical egg transfer in mares. J Reprod Fertil 41:313–320
Ombelet W, van Robays J (2015) Artificial insemination history: hurdles and milestones. Facts Views Vis Obgyn 7:137–143
Orland B (2017) The invention of artificial fertilization in the eighteenth and nineteenth century. Hist Philos Life Sci 39:11
Perry G (2017) 2016 statistics of embryo collection and transfer in domestic farm animals. IETS Newsletter 37:8–18
Petersen B, Niemann H (2015) Molecular scissors and their application in genetically modified farm animals. Transgenic Res 24:381–396
Pincus G (1930) Observations on the living eggs of the rabbit. Proc R Soc Lond B 107:132–167
Pincus G, Kirsch RE (1936) The sterility in rabbits produced by injections of oestrone and related compounds. Am J Phys 115:219–228
Polge C (2007) The work of the animal research station, Cambridge. Stud Hist Philos Biol Biomed Sci 38:511–520
Polge C, Smith AU, Parkes AS (1949) Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 164(4172):666
Prather RS, Barnes FL, Sims MM et al (1987) Nuclear transplantation in the bovine embryo: assessment of donor nuclei and recipient oocyte. Biol Reprod 37:859–866
Rothschild MF, Plastow G (2014) Applications of genomics to improve livestock in the developing world. Livest Sci 166:76–83
Schafberg R, Swalve HH (2015) The history of breeding for polled cattle. Livest Sci 179:54–70
Schriver MD, Kraemer DC (1978) Embryo transfer in the domestic feline. Am Ass Lab Anim Sci Publ 78–4:12
Schultz H (1980) History of the international embryo transfer society II. Theriogenology 13:7–11
Shendure J, Balasubramanian S, Church GM et al (2017) DNA sequencing at 40: past, present and future. Nature 550:345–353
Sims M, First NL (1994) Production of calves by transfer of nuclei from cultured inner cell mass cells. Proc Natl Acad Sci U S A 91:6143–6147
Spemann H (1938) Embryonic development and induction. Yale University Press, New Haven, CT, pp 373–398
Steptoe PC, Edwards RG (1978) Birth after reimplantation of a human embryo. Lancet ii:366
Sunderam S, Kissin DM, Crawford SB, Folger SG, Boulet SL, Warner L, Barfield WD (2018) Assisted reproductive technology surveillance – United States, 2015. MMWR Surveill Summ 16:1–28
Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
Telugu BP, Donovan DM, Mark B et al (2016) Genome editing to the rescue: sustainably feeding 10 billion global human population. NIB J. https://doi.org/10.2218/natlinstbiosci.1.2016.1743
Telugu BP, Park KE, Park CH (2017) Genome editing and genetic engineering in livestock for advancing agricultural and biomedical applications. Mamm Genome 28:338–347
Thibier M (2008) Data retrieval committee statistics of embryo transfer – year 2007. IETS Newsletter 26:4–9
Tsunoda Y, Yasui T, Shioda Y et al (1987) Full-term development of mouse blastomere nuclei transplanted into enucleated two-cell embryos. J Exp Zool 242:147–151
Venter JC, Adams MD, Myers EW et al (2001) The sequence of the human genome. Science 291:1304–1351
Vishwanath R (2003) Artificial insemination: the state of the art. Theriogenology 59:571–584

Wakayama T, Perry ACF, Zuccotti M et al (1998) Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 394:369–374
Wang GD, Xie HB, Penb MS et al (2014) Domestication genomics: evidence from animals. Annu Rev Anim Biosci 2:65–84
Warwick BL, Berry RO (1949) Inter-generic and intra-specific embryo transfers in sheep and goats. J Hered 40:297–303
Warwick BL, Berry RO, Horlacher WR (1934) Results of mating rams to Angora female goats. In: Proc 27th Ann Meet Am Soc Anim Prod, pp 225–227
Weissmann A (1893) The germ-plasm: a theory of heredity. Translated by W. Newton Parker and Harriet Rönnfeldt. Scribner, New York, NY
Whittingham DG (1971) Survival of mouse embryos after freezing and thawing. Nature 233:125–126
Willadsen SM (1986) Nuclear transplantation in sheep embryos. Nature 320:63–65
Willett EL, Black WG, Casida LH et al (1951) Successful transplantation of a fertilized bovine ovum. Science 113:247
Wilmut I, Rowson LE (1973) Experiments on the low-temperature preservation of cow embryos. Vet Rec 92:686–690
Wilmut I, Taylor J (2018) Cloning after Dolly. Cell Reprogram 20:1–3
Wilmut I, Schnieke AE, McWhir J et al (1997) Viable offspring derived from fetal and adult mammalian cells. Nature 385:810–813
Zeder MA, Bradeley DG, Emshwiller E et al (2006) Documenting domestication: new genetic and archaeological paradigms. University of California Press, Berkeley/Los Angeles, CA