The Sheep's Role in History (Part 2)

By Debra K. Aaron, Professor, Department of Animal and Food Sciences, University of Kentucky

In Part 1 of "The Sheep's Role in History" (HoofPrint, Volume 21, Fall 2015) you read the story of Robert Bakewell, the "Father of Animal Breeding." It was his revolutionary visions that led to important progress in the selective breeding of livestock and fortified the sheep's role in genetic history. In Part 2, we fast-forward to the 20th century and beyond. It is now a world where genetic engineering, biotechnology and genomics are the tools for genetic improvement of livestock. It is also where we tell Dolly's story.

Genetic Engineering in Livestock: A Long History

By all accounts, sheep and goats were the first livestock species to be domesticated. This occurred approximately 11,000 years ago and represents the first human manipulation of genes, which, once again, put the sheep at the forefront of genetic history. This first "genetic engineering" was accomplished not in test tubes or petri dishes but through artificial selection and traditional animal breeding techniques. Such techniques included inbreeding and crossbreeding. In addition, diverse strains of animals were sometimes crossed to produce greater genetic diversity. Consider the equine family. Over the past 3,000 years, mares have been bred with jacks to produce mules and stallions have been bred with jennies to produce hinnies for use as work animals. This technique continues to be used today and represents an early form of genetic engineering.

The modern era of genetic engineering, with manipulation of genes and DNA in test tubes and petri dishes, began in 1953 when American biochemist James Watson and British biophysicist Francis Crick presented their double-helix model of DNA (Figure 1). This was followed by Swiss microbiologist Werner Arber's discovery

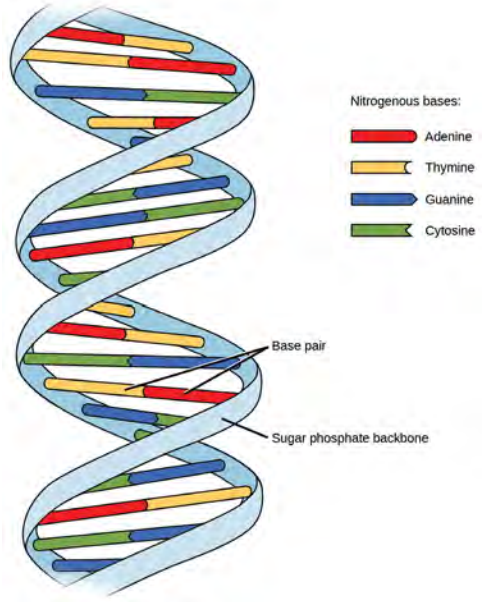

Figure 1. Double Helix DNA (http://commons.wikimedia.org/wiki/File:229_Nucleotides-01.jpg).

of special enzymes, called restriction enzymes, in bacteria. These enzymes can cut DNA strands of any organism at precise points. In 1973, American geneticist Stanley Cohen and American biochemist Herbert Boyer removed a specific gene from one bacterium and inserted it into another using restriction enzymes. This event marked the beginning of biotechnology. Then in 1977, genes from other organisms were transferred to bacteria, an achievement that eventually led to the first transfer of a human gene.

Animal biotechnology today is based on the science of genetic engineering. Under the umbrella for genetic engineering exist other technologies, such as transgenics and cloning. This brings us to Dolly's story.

Hello Dolly

Her Birth. Dolly, a Finn-Dorset ewe, was born on July 5, 1996, at the Roslin Institute in Edinburgh, Scotland. She made genetic history because she was the world's first mammal to be successfully cloned from an adult somatic (body) cell. This is one of the most significant scientific breakthroughs ever because her birth and subsequent survival proved that adult cells could program themselves into a new being. Today, Dolly is a scientific icon, a

Hello Dolly (AP Photo/Paul Clements)

household name. Much of her story comes from accounts reported by The Roslin Institute (http://www.roslin.ed.ac.uk/public-interest/dolly-the-sheep/).

Dolly started her life in a test tube. The technique Keith Campbell, Ian Wilmut and their Roslin Institute colleagues used to create her is called nuclear transfer. It involves transferring the nucleus from a diploid cell (which contains the full complement of an individual's chromosomes) to an unfertilized egg from which the maternal nucleus has been removed. Figure 2 shows how it worked in Dolly's case. An adult Scottish Blackface ewe (the cytoplasmic donor) provided an unfertilized egg cell, an ovum. Scientists removed the nucleus of this egg leaving only the cytoplasm. Cytoplasm is the thick solution that fills the egg and is enclosed by the cell membrane. It is mainly composed of water, salts and proteins. Concurrently, an adult mammary cell was taken from the udder of a 6-yr-old Finn-Dorset ewe (the DNA donor). Then, all the cellular machinery was stripped away, leaving only the cell nucleus, which contained DNA and all the genetic material needed to create life. Next, these scientists injected the nucleus into the "empty" egg cell. The two cells were fused using a jolt of electricity. The fused (or hybrid) cell began to divide and develop into a blastocyst (the early stage of an embryo). Once normal development was confirmed at 6 days, the embryo, that was to become Dolly, was implanted into a surrogate (recipient) ewe. The surrogate was another Scottish Blackface ewe. When the resulting clone was born, after a normal, full-term pregnancy, she was

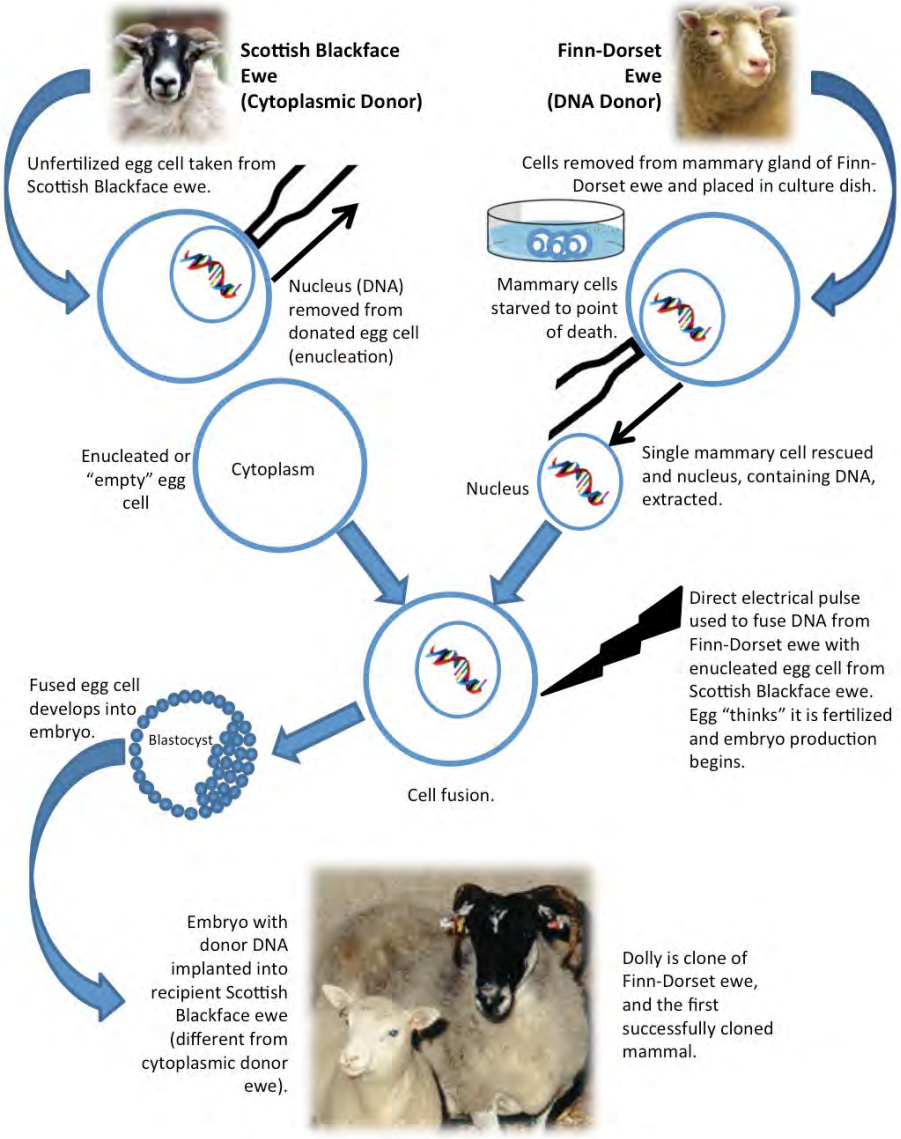

Figure 2. How Dolly the Sheep was Cloned. Photo Credits: Top Left (www.scottish-blackface.co.uk/); Top Right (www.abc.net.au/news/2013-05-16); Bottom left (www.coldmeadow.com/dolly/ddstory/story.html)

named Dolly, in honor of the singer Dolly Parton, because it was a mammary cell that was cloned.

Dolly was an immediate superstar. The press flocked to Roslin Institute and the University of Edinburgh after her birth was announced. Although most of the Roslin staff thought Dolly's initial stardom would be brief and quickly fade, this was not the case. The press from all over the world continued to visit Dolly for the rest of her life, with their interest peaking any time there was a concern over her health.

Her Life. The goal was to manage Dolly as a normal sheep. To that end, she was allowed to reproduce like any other ewe. She was bred to a small Welsh Mountain ram over three mating seasons and successfully produced six lambs. A single ewe lamb, named Bonny, was born in the spring of 1998. Twins followed the next year and triplets the year after that.

Dolly started suffering from ill health in 2002, when she was diagnosed with a form or arthritis. Then a year later, she developed a progressive lung disease and a decision was made to have her put down. The causes of her health issues were never discovered and, although never proven, there is still widespread speculation that she died prematurely as the result of being a clone.

Her Legacy. Dolly has been called "the world's most famous sheep." In hindsight, Dolly the clone was just a sheep. She was not produced with the intention of widespread cloning of livestock for conventional production. She was actually created as part of research into producing medicines in the milk of farm animals. Nonetheless, her birth completely changed the field of genetics and science as a whole. Although she died prematurely, her life left a lasting legacy. First, she existed. The creation of a viable clone of a complex mammal was unexpected. In fact, her birth was the end result of more than 250 attempts at cloning a sheep. Second, Dolly's birth overturned the assumption among scientists that the whole process of differentiation was irreversible. We all start life as a single cell, the fertilized egg. The cell divides and multiples and by the time we are born, there are maybe 200 different cell types, each containing the same DNA, the same 30,000 or so genes, but each differentiated into a specific role. A cell might become a nerve cell, a muscle cell or a mammary cell, for example. Until Dolly, scientists thought this was a one-way process.

Dolly showed that it was possible to take an adult differentiated cell, that is a mature body cell that has reached the end of its developmental stages, and essentially turn its clock back, to reactivate all its genes and make the cell behave as though it was a fertilized egg. In Dolly's case, a mammary cell reverted back to its embryonic state. Since Dolly, there have been many more cloned animals and the technology continues to improve.

What is the long-term significance of Dolly? That's difficult to say. Practical implications of cloning livestock are limited because of costs, governmental regulations and consumer fears. Dolly's longer lasting legacy will likely be in our understanding of development and genetics. Our understanding now is that the cells in our bodies are a lot more plastic and changeable than previously thought. The technology used to create Dolly may offer hope for the process of therapeutic

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cloning, where new healthy cells can be created from differentiated cells that have been wound back to their embryonic state. If a "blank" cell has the potential to grow into any other cell, it could be used to mend aging, diseased and damaged tissues and organs.

Pulling the Wool off the Sheep Genome

An animal's complete set of DNA is called its genome and virtually every cell in its body contains a copy. Genomics, then, is the term coined by scientists that describes the large-scale sequencing and analysis of DNA. You might ask, "What is the difference between genetics and genomics?" The key distinguishing feature between the two is that genetics investigates the activity and composition of a single cell and genomics includes all genes and their associations in order to recognize their collective influence on the development and growth of an animal. Genomics is a rapidly expanding field in animal agriculture, and sheep have played a role in that development. Through genomics, scientists aim to determine complete DNA sequences and perform genetic mapping to help understand the relationships between genes and important traits, such as growth, carcass composition and disease or parasite resistance.

As noted earlier, the sheep was one of the first animals to be domesticated. It is, however, one of the last to have its complete DNA sequenced. Goats, cattle, pigs and horses all beat the sheep in having their genomes read. Finally, in 2014 the sheep joined their ranks when its complete genetic code was published in the June 6 Science magazine (Volume 244, Number 6188, Pages 1168-1173). This was the culmination of eight years of work by an international team of scientists. Sheep lagged behind the other species, not because of their uniqueness, but because there was less international industry support to help fund the project. Eventually, Australian scientists formed a group with scientists from New Zealand, the United States, Europe, and China, called the International Sheep Genomics Consortium, to read the genome of two Texel rams.

Mapping the sheep's genetic code has revealed mysteries associated with wool and forage digestion. By comparing the sheep genome with cattle, goat and pig genomes, this group of scientists were able to identify several genes that are associated with wool production. They also discovered genes that are important in the evolution of the rumen, a chamber in the sheep's stomach that breaks down cellulose-rich plants like grass into protein. Finally, an understanding the complete genetic code has the potential to further development of DNA testing and to speed-up the process of selection, which will help producers improve their flocks.

The Ram that Pulled the Wool Off the Sheep Genome (http://phys.org/news/2014-06-sheep-genome-goats.html)

What Does the Future Hold?

A better understanding of the genetic makeup of sheep will lead to improvements in efficiency of production of meat, wool and milk. In addition, it will contribute to healthier sheep through better management of diseases and parasite infections. Use of genomic information already allows direct assessment of genetic merit of potential breeding animals for simply inherited genetic defects and for using genes with large effects on prolificacy and disease resistance. Use of genomic information to aid genetic improvement in quantitative traits, such as growth, feed efficiency and carcass composition, is progressing more slowly, but it is progressing. Continued progress will depend on organized efforts by research and industry to record performance, calculate estimates of genetic merit and determine genome profiles on large numbers of sheep.

We have come a long way since Robert Bakewell's time but there is still much to learn!

Dr. Debra K. Aaron, PhD, *professor in the UK Dept. of Animal Sciences, teaches animal science and genetics. Her research interests are in sheep breeding and genetics.*