PART I THE SCIENCE OF CLONING

THE YEAR WAS 1997. THE NEWS WAS AMAZING: a team of Scottish scientists that included Ian Wilmut and Keith Campbell had cloned a lamb from an adult sheep. Although Dolly had been born on July 5, 1996, the team had delayed the announcement to protect their ability to publish their work,[1] which they did nearly eight months later in the science journal *Nature*.[2]

Dolly promptly became a media sensation. The outpouring of scientific research, academic analysis, and public discussion makes it likely that the reader already possesses some information (or misinformation) about this first and most famous mammal cloned from an adult cell. Also, the experiment that produced her serves as a basis for discussing later developments in cloning science. For these reasons, Part I will begin the discussion with a review of how Dolly was made and what we know about her.

A. The Story of Dolly

Dolly's story begins with cells harvested from the mammary glands of a long-since deceased ewe of the Finn Dorset breed.[3] The cells were retrieved from storage and placed in culture. Each cell in the culture contained in its nucleus the nuclear deoxyribonucleic acid (DNA) of the donor ewe, arrayed in structures known as chromosomes. Embedded within those chromosomes were the donor's genes. Genes

are discrete DNA segments that specify the sequence of amino acid chains[4] that form the building blocks of proteins. Proteins are major structural molecules that form single cells; groups of cells assemble to create larger tissues; and collections of different tissues form the body of an organism.[5]

Thus, all the information necessary to make a sheep was present in each individual cell in the culture. However, most of the genes in those cells had been switched off during the process of differentiation, through which embryonic cells diverge in function and take on specialized roles in the body.[6] Only genes necessary to the function of mammary gland cells were still active in the cultured cells. Wilmut and his team had to find some means of reprogramming the gene expression of these donor cells so that the nuclear DNA could support the development of a new organism.[7]

To accomplish this feat, the scientists relied upon the magic of the egg, which contains factors that promote the replication of DNA.[8] The scientists retrieved eggs from Scottish Blackface ewes (a different breed than the Finn Dorset cell donor) and removed the chromosomes, leaving the eggs without any DNA of their own (a process often referred to as *enucleation*).[9] Meanwhile, the scientists readied the mammary gland cells for reprogramming. They deprived the cells of the nourishment needed to grow and proliferate, starving them into a quiescent state.[10]

The next challenge was to transfer DNA from the mammary gland cells into the empty, waiting eggs. This transfer was accomplished in two steps: first, one scientist passed a donor cell into an egg via pipette; second, another used electrical pulses to fuse the donor cell to the egg and simultaneously activate that egg so that it could begin the reprogramming process and initiate development of a cloned lamb.[11]

Wilmut and his team obtained 277 fused couplets from this process. Twenty-nine of those developed into embryos and were transferred into thirteen surrogate ewes. One Scottish Blackface ewe became pregnant and delivered Dolly, a healthy lamb. In terms of lambs born per embryos transferred, the experiment had a birthrate of 3.4 percent.[12]

Dolly did not share the breed of her egg donor or surrogate mother. In appearance, she was a Finn Dorset sheep like her cell donor.[13] DNA analysis further confirmed she was cloned from the cell donor.[14]

This accomplishment was striking for a number of reasons. Ordinarily, mammals conceive new life through the combination of *germ cells*, that is, sperm and eggs. Dolly had been conceived using a non-germ, or *somatic cell* (which explains why cloning is sometimes called somatic cell nuclear transfer). Her method of conception was asexual, rather than sexual. Another happy surprise was the success in reversing the differentiated state of the adult donor cell. Although Wilmut and Campbell had already cloned sheep from embryo-derived cell lines,[15] biologists had long believed that the cloning of a mammal from an adult cell was impossible.[16]

Granted, the experiment was inefficient, and that raised eyebrows. Moreover, many politicians and reporters misunderstood the 276 fused couplets that either never became embryos or failed to produce a pregnancy, viewing them as deformed or dead lambs.[17] This, however, is incorrect. There was only one pregnancy derived from adult cells, and that resulted in the birth of a healthy lamb (Dolly).[18] Moreover, Dolly stayed healthy as she matured. Defying those who questioned her fertility, she gave birth to six healthy lambs conceived the old-fashioned way.[19]

Still, skeptics questioned whether she could possibly be normal, and the media eagerly reported every indication that she might not be. For example, after she turned one year old, the Wilmut team measured her telomeres (repetitive DNA sequences that protect the ends of chromosomes against degradation)[20] and found them to be shorter than normal for a sheep of her age.[21] Noting that the difference could have been due to natural variations among sheep, the team pointed out that it was not certain that telomere length reflected physiological age. In fact, at age one she was healthy and typical for a sheep of her breed.[22] However, the team also stated that Dolly's telomere length was consistent with the age of her DNA donor (who had been six years old when the mammary

gland cells were harvested) and the length of time the cells had been cultured prior to her cloning.[23] This captured the attention of the media, which reported that Dolly had been born prematurely old.[24]

When Dolly developed arthritis in her left hind leg at age five, critics again raised the specter of premature aging, discounting the explanation that Wilmut gave: she was a celebrity and stood on her hind legs to beg for treats and attention from visitors.[25] When she was euthanized at age six after contracting a contagious lung disease that was spreading among the sheep in her barn, reporters wrote premature aging into their obituaries.[26]

The idea that a lamb had been born six years old blurred the distinction between Dolly and her DNA donor. It reinforced the popular misconception that cloning was a means of resurrecting the dead. Even people who realized that resurrection was impossible had a tendency to conceptualize Dolly as a copy of the DNA donor. It was easy to forget that she had been born as a lamb because the reports of her birth (and accompanying photos) came much later, after she was nearly eight months old. The absence of the sheep that had donated the DNA made it impossible to draw comparisons that would have revealed her unique characteristics and individuality.[27]

B. Updating the Science of Cloning

Dolly was born sixteen years ago. Since then, cloning has proven to be a popular field, attracting interest from scientists all over the world. These scientists have conducted countless experiments in many species, including our own. Accordingly, the goal of Part I is simple: to bring the science of cloning up to date for the reader.

Chapter 1 discusses methods that have been used to clone animals, and the efficiency rates that scientists have obtained. It also examines the health status and function of cloned animals.

Chapter 2 shifts the focus to the individuality of animals born through cloning. Applying basic biological principles and experimental results,

the chapter explains why these animals often do not look or act the same as the animals that donated the DNA for the cloning procedure. It also discusses scientific evidence showing that animals born through cloning are not born old. Rather, they start life as infants and enjoy healthy lifespans.

Chapter 3 carries the discussion into the human realm. It explains that human embryos have been cloned for research purposes but no babies have been born thus far. The rest of the chapter uses biological principles and animal experiments to predict the characteristics of humans born through cloning.

1 Animals Born Through Cloning Are Ordinary Members of Their Species

THE CREATION OF DOLLY THE SHEEP RAN CONTRARY TO biological dogma. Some scientists questioned at first whether Ian Wilmut and Keith Campbell had truly cloned her from the somatic cell of an adult animal.[1] Fifteen years later, scientists now realize that Dolly was the vanguard of her kind.

A. Which Species Have Been Cloned?

Working with differentiated cells taken from young and mature animals, scientists have cloned a menagerie: livestock such as cattle, sheep, goats, and pigs[2]; experimental animals such as mice, rats, and rabbits[3]; and pets such as dogs, cats, and horses.[4]

Cloned species are not limited to familiar domesticated animals. Scientists have also turned the technology to more exotic uses. Although no dinosaurs have been cloned (apologies to *Jurassic Park* fans), some labs have cloned other species that are threatened or extinct. For example, South Korean researchers have cloned the gray wolf,[5] which is still considered endangered in some parts of the United States.[6] An American team has cloned a gaur, a type of wild ox that is on the brink of extinction.[7] Some Japanese researchers are even attempting to clone the extinct wooly mammoth.[8]

There is one notable gap in the menagerie: not a single primate has been cloned from a live animal.[9] However, that momentous event may

be drawing closer. In 2007, Oregon researchers took DNA from an adult rhesus monkey, cloned dozens of embryos, and used the embryos to create two stem cell lines.[10] This accomplishment hints that cloned baby monkeys may not be far behind.

1. Which Species Are Most Commonly Cloned?

Scientists and researchers clone animals from certain species far more than others. Approximately 75 percent of cloning labs work on livestock. Cattle lead the herd, serving as research subjects in nearly half of all cloning labs.[11] Pigs are also popular, functioning as research subjects in approximately 15 percent of cloning labs.[12] The main reasons for this interest are commercial. The food industry wants to replicate individual animals with desirable genetic traits – not so that they can be eaten, but so that they can sire offspring.[13]

The biomedical industry is another player. It is interested in *transgenic cloning* – that is, cloning animals from cells that have first been altered in the lab by adding or inactivating genes. One of the first transgenic cloned animals was a sheep named Polly. Ian Wilmut cloned her from DNA to which he added a human gene. Her milk was modified to contain human factor IX, a clotting protein used to treat hemophilia.[14] Similarly, transgenic cloned cows may one day produce milk laced with proteins that can be used in medical therapy.[15] Other scientists have engineered cloned pigs. Their goal is to create hearts and other transplantable organs that the human immune system will not reject.[16]

Moving beyond livestock, one finds that cloning is more than a commercial activity; it is also a valuable research tool. Scientists can use cloning experiments to investigate fundamentals of cell biology, genetics, and epigenetics.[17] Mice are useful research animals because they reproduce rapidly and prolifically and have relatively short life spans.[18] Thus, it is not surprising that mice are research subjects in nearly one-fifth of labs; only cattle are cloned in more labs.[19]

B. How Efficient Is Animal Cloning?

The original Dolly experiment was not very efficient. Only one of the twenty-nine embryos transferred to surrogate mother sheep resulted in the birth of a lamb. In other words, the birthrate was only 3.4 percent.[20]

In 2010, Dr. Keith Campbell made headlines by revealing that he had cloned again from the same stock of frozen mammary gland tissue that had produced Dolly. Improvements in cloning technology resulted in four healthy sheep born in 2007. This time, one out of every five embryos transferred to surrogate mother sheep resulted in the birth of a lamb.[21] In other words, the birthrate was an impressive 20 percent. That increased rate is a big leap forward from the original Dolly experiment.

Further details on these sheep were not available at the time this book went to press. However, in the past fifteen years, scientists have conducted hundreds of experiments involving various species. These scientists have utilized different cloning methods. Their success in generating animals has also varied.

This book presents data on the two animal species that are most commonly cloned: cattle and mice.[22] Although cloning experiments can be rather technical, it is worth examining the data for what it can reveal on the methods and efficiency of cloning.

1. Cattle

Researchers working with cattle most commonly clone from *fibroblasts* – that is, a type of progenitor cell that is not yet terminally differentiated and can develop into bone, cartilage, fat, or muscle.[23] Early pregnancy rates can be high: as many as 65 percent of embryos transferred lead to a pregnancy. This rate is comparable to early pregnancy rates achieved through bovine in vitro fertilization (IVF). After seven weeks of gestation, however, few IVF fetuses miscarry while most cloned fetuses do.[24] Judging by reports in the literature, approximately 9–20 percent of cloned embryos transferred to the womb produce live-born calves

delivered at term after nine months gestation.[25] This birthrate falls short of the nearly 40 percent birthrate achieved in bovine IVF.[26] Why is cattle cloning less efficient than IVF?

a) Genetic Abnormalities

Genetics offers one possible explanation. A cloned embryo will not develop properly if there are errors in its genetic code. For example, suppose the nuclear DNA in the donor cell mutates while the cell is still in the body of the donor. Or, suppose the nuclear DNA is fine when the donor cell is harvested from the donor but mutates in a Petri dish while it awaits transfer to an egg.

However, experiments have not yet shown that mutations are a major obstacle to cattle cloning. Indeed, the evidence we have cuts the other way. Cattle have been successfully cloned from cells taken from an older donor and cultured for several months; the birthrate was comparable to rates obtained in experiments utilizing cells harvested from younger animals and cultured for shorter periods of time.[27]

b) Reprogramming

Other biological barriers to cloning involve not genetic but rather *epigenetic factors* – that is, heritable changes that go beyond alterations in DNA sequence.[28] For example, epigenetic modifications switch genes on and off, leading to the differentiation of cells.[29] Such modifications make it a challenge to clone from the nuclear DNA of an adult cell. The egg must remove the modifications so that all the genes are once again available for expression.[30] In other words, the egg must reprogram the nuclear DNA of the cell.

Some scientists believe that animal cloning is inefficient because of inadequate reprogramming. To test this hypothesis, one group of researchers studied the gene expression profiles of cloned bovine embryos. To their surprise, the profiles for cloned embryos were quite similar to those of embryos created through artificial insemination. Less than 1 percent of the more than 5,000 genes studied were differentially expressed.[31]