

Expression of Human Clotting Factor IX by Cloned Sheep (1997)

In the 1990s, researchers working at the Roslin Institute in Edinburgh, Scotland, performed cloning experiments in collaboration with PPL Therapeutics in Roslin, Scotland, on human coagulation factor IX, a protein. The team of scientists used the methods identified during the Dolly experiments to produce transgenic livestock capable of producing milk containing human blood clotting factor IX, which helps to treat a type of hemophilia. By using a cell's resting state, called quiescence, or G0, and transferring modified nuclear material from one cell to an egg cell that had had its nuclear material removed, the researchers developed a method to produce genetically modified mammals, including humans. Angelika E. Schnieke, Alexander J. Kind, William A. Ritchie, Karen Mycock, Angela R. Scott, Marjorie Ritchie, Ian Wilmut, Alan Colman, and Keith H. S. Campbell published the results of their experiments as "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts" (hereafter called "Human Factor IX"). The article details the methods that produced the cloned sheep named Polly, as well as other cloned and genetically altered sheep.

In 1985, Robert E. Hammer's group at University of Pennsylvania in Philadelphia, Pennsylvania, produced animals that expressed genes from other species, called transgenic animals. Scientists genetically manipulated the animals in a process called pronuclear microinjection. In this technique, scientists inject DNA containing the desired gene into the nucleus of the egg, called a pronucleus, just before the pronuclei of the egg and sperm combine during fertilization. Some of the resulting animals are born with the injected genes expressed, but the success rate is low. Genetic material can also recombine in such a way that it becomes a detriment to the organism, or in such a way that not all of the cells in the organism have the gene.

In 1996 research on sheep embryos at the Roslin Institute, prior to the "Human Factor IX" experiment, established the claim that genetic alterations on adult body or somatic cell nuclei were possible. The researchers found that cloned sheep could express genes from another species by transferring altered nuclei into embryonic cells. The 1997 article "Viable Offspring Derived from Fetal and Adult Mammalian Cells" by Wilmut, Schnieke, and Jim McWhir, Kind, and Campbell reported the earlier sheep embryo experiments.

Other information for "Human Factor IX" came from the 1996 experiment, "Sheep Cloned by Nuclear Transfer from a Cultured Cell Line." Campbell, McWhir, Ritchie, and Wilmut cloned sheep from embryonic cells grown in a laboratory in 1996. In that experiment, the researchers established that if they transferred genetic material into a cell that was in quiescence, then they could more efficiently clone the cells.

Before coming to the Roslin Institute, Schnieke studied the modes of transmission for RNA viruses, the production of cross-species genetic modification, or transgenic animal models, and the deactivation of specific genes from producing proteins. Kind was Schnieke's husband and worked at PPL Therapeutics. Ritchie specialized in micromanipulation procedures, or the ability to work with small tools and specimens under a microscope. He had previously performed procedures in other sheep embryo experiments, and he had helped with the same techniques that enabled the cloning of Dolly the sheep in 1996. Mycock was a technician who helped with embryological manipulation procedures in the lab with Ritchie. Scott was a technician working for PPL Therapeutics. Scott multiplied the cell cultures needed for the experiment. Marjorie Ritchie, William Ritchie's wife, organized the surgeries needed to implant the sheep embryos. Lastly, Wilmut researched animal genetics and was part of various animal embryo experiments at the Roslin Institute. Coleman was research director

of the Roslin Institute at the time the Dolly and Polly experiments. Campbell studied microbiology and had worked previously with reprogramming cell nuclei as well as transplantation of nuclear material in other experiments at the Roslin Institute.

The team of scientists used the methods identified during the Dolly experiments to produce transgenic livestock capable of producing milk containing human blood clotting factor IX, which helps to treat a type of hemophilia. Scientists had produced transgenic livestock since 1985, but only about five percent of these animals expressed a transgenic DNA, or transgene, in their own genomes. Among these animals, few of the transgenes passed naturally to offspring. The researchers claimed that it is simpler to incorporate foreign DNA into adult cell nuclei as opposed to juvenile nuclei, a process called transfection. The scientists, first transfected adult cells and then used the methods introduced from cloning Dolly to transfer the modified nuclei into awaiting enucleated oocytes, and then they cloned a transgenic sheep.

The scientists used donor nuclei from cells, which the scientists said were fetal fibroblast cells, of thirty-five-day-old fetuses of a Poll-Dorset sheep. The donor nuclei, modified to be immune to the antibiotic neomycin, enabled the scientists to check that the cells in fetal sheep were from the implanted embryo. Immunity to neomycin would also identify the cells modified to have the transgenes. The researchers used a method called lipofection, which uses the chemical lipofectamine to penetrate the outer membrane of cells, to insert the DNA into these cells. Some fetal fibroblasts were non-transfected and used as controls.

The scientists deprived the cells of growth factors and induced them into a state of quiescence. The scientists then found that even though the cells had been transfected, they could withstand the lack of growth factors and resumed the cell cycle without damage after the reintroduction of the growth factors. The researchers transplanted four types of nuclei into enucleated egg cells: male nuclei without transfection, female nuclei without transfection, female nuclei with transfection of the FIX gene at less than five copies, and female nuclei with the transfection of the FIX gene with more than ten copies.

The researchers then transplanted the embryos into Scottish Blackface sheep, examined the pregnancies, and recorded the resulting births. Of the lambs born, there were one non-transfected male, three non-transfected females, two transfected females with more than ten copies of the gene, and one transfected female with less than five copies of the gene. One of the female transfected lambs expressed the gene products at a particularly high level. The lamb was cloned from the Poll Dorset breed of lamb and was therefore name Polly. Each of the transgenic lambs was completely transgenic and not partially transgenic. A partially transgenic lamb would contain only a few cells that express the gene. Sheep born from microinjection techniques often are partially transgenic. Cloning produced one transgenic lamb per 20.8 sheep, as opposed to one transgenic lamb produced per 51.4 sheep by microinjection. The experiment indicated that cloning procedures produce a greater number of transgenic lambs than do microinjection techniques.

The scientists found some of the births problematic. The gestations of all of the lambs were longer than the normal gestational term for this particular breed of sheep. There were no increases in mortality rate or disease, in the ewes however, a result that Wilmut indicated was important in understanding that genetic modification does not increase death rate.

Outside the research team, scientists questioned whether transgenic animals were capable of reproducing and having offspring that contained the same genes. Eventually the transgenic sheep reproduced and had offspring with the same FIX protein present in the cells.

According to Wilmut, animal activists advocated against and misunderstood this experiment. Some activists claimed that Polly contained human characteristics. Wilmut explained that while Polly's genome contains a human gene, it is only one gene out of thousands of normal sheep genes.

The "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts" experiment successfully produced a sheep that can express a human gene resulting in a human protein. Quiescence and nuclear transfer improved the ability to produce transgenic animals.

Sources

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