

In 1995 and 1996, researchers at the Roslin Institute in Edinburgh, Scotland, cloned mammals for the first time. Keith Campbell, Jim McWhir, William Ritchie, and Ian Wilmut [5] cloned two sheep [6], Megan and Morag, using sheep [6] embryo cells. The experiments indicated how to reprogram nuclei from differentiated cells to produce live offspring, and that a single population of differentiated cells could produce multiple offspring. They reported their results in the article "Sheep Cloned by Nuclear Transfer from a Cultured Cell Line" in March 1996. This experiment led the Roslin team to later clone mammals from adult body cells and to genetically engineer mammals.


The scientists tested two hypotheses in the sheep experiments from 1994 to 1995. The first hypothesis was Jim McWhir's, who said that to have embryos created from nuclear transfer, it was necessary to have nuclei that could produce cells that could develop into any cell type, called totipotent cells. McWhir hypothesized that researchers could culture totipotent sheep cells. The second hypothesis, presented by Campbell, was the claim that reprogrammed differentiated cells could be totipotent. Campbell suggested that to use nuclei from differentiated cells for cloning, scientists only had to ensure that the cytoplasm from the receiving egg cell and the donor nucleus were in quiescence.

The scientists selected four groups of female sheep (ewes). Using two distinct breeds of sheep ensured that the offspring produced were the result of cloning and not the result of eggs fertilized within the oviduct. Scottish Blackface sheep provided the oocytes, which had nuclei removed (enucleated) and which became the receiving enucleated oocytes. These sheep constituted the first of the four groups used. The scientists obtained the oocytes by injecting ewes with a follicle-stimulating hormone, called gonadotropin-releasing hormone, which facilitates the release of oocytes from the ewes' ovarian follicles. The scientists flushed out the oocytes from the ewes' fallopian tubes with a saline solution then collected the oocytes.

Ritchie removed the nucleus from each oocyte under a microscope by extracting the nucleus with a glass pipet. Scientists confirmed enucleation by placing donor oocytes in calcium-free media containing a chemical that fragments DNA, Cytochalasin B, and a fluorescent dye that specifically stains DNA, Hoechst 33342, and looked at the glass pipet under ultraviolet light. If the pipet glowed due to the fluorescent dyes present in the DNA, and if the cytoplast, or inner part of the cell, did not glow, then enucleation was successful. In 1994, the cytoplasts used were all low in MPF protein, thus ensuring the cytoplasts could accept nuclei. In 1995, the scientists divided the cytoplasts into three different MPF protein groups. Cytoplasts in the first group had low amounts of MPF protein, the second group had high amounts of MPF protein at first, but declined to low MPF after embryo fusion, thus emulating natural fertilization, and the third group had high amounts of MPF protein both pre- and post-embryo fusion.

The scientists used two processes to obtain nuclei (karyoplasts) to implant into the enucleated oocytes. The first used McWhir's theory of deriving totipotent cells from nine-day-old embryos, which had formed into an embryo disc. The researchers placed these cells in a medium that contained nutrients as well as a protein that discourages cell differentiation but not cell multiplication, the protein leukemia inhibition factor (LIF). The researchers placed the embryo disc in an incubator for a few days then removed the cells from the medium and divided them into four new cultures. This process is called a passage, meaning the cells were passed into a new medium. McWhir and his colleagues continued for thirteen passages. By the fourth passage, the cells began to differentiate, despite the presence of LIF proteins in the medium. The scientists named the cells that maintained totipotency after thirteen passages totipotent for nuclear transfer, or TNT4. Accordingly, they called this portion of the experiment the TNT4 section.

Next, the scientists tested Campbell's hypothesis called the cell-cycle step of the procedure. Campbell's hypothesis was that they could reprogram diploid cells nuclei that were in a state of quiescence by placing them into a cytoplasm with high concentrations of MPF protein. Campbell said that cells deprived of growth factors entered quiescence. Welsh Mountain sheep
provided the karyoplasts for the testing of Campbell's hypothesis. The experimenters held cytoplasts in place with a glass pipet. Using another glass pipet, Ritchie inserted the karyoplasts into cytoplasts through the hole created from enucleated cytoplasts. The researchers then placed combinations of cytoplasts and karyoplasts between two electrodes and fused them together. To do so, they used electrical fusion methods developed by Steen Willadsen [25] at the British Agricultural Research Council's Institute of Animal Physiology at Cambridge, UK, in the 1980s. A gelatinous solution obtained through boiling algae (agar) coated over the fused embryos protected them from environmental factors between transfers.

The scientists injected a third group of ewes with gonadotropin [20] releasing hormone [19] to simulate pregnancy [26]. They surgically implanted the altered embryos into the ewes. These sheep [6] were temporary holders of the embryos. The ewes incubated the embryos in their oviducts until the embryos began to differentiate and contain an inner cell mass [27], called blastocysts. The scientists then removed the blastocysts and examined them under a microscope [11] to ensure development had begun.

The scientist's implanted two blastocysts, one into each uterine horn, of the surrogate [28] Scottish Blackface ewes of the fourth group. Those ewes gave birth after about one hundred and forty-seven days. The researchers monitored the ewes to ensure there were no complications. Of the forty-seven embryos from TNT4 blastocysts, six were born. Two resulted from a sixteen-cell blastocyst [29], one resulted from a first passage blastocyst [29], one from a second passage blastocyst [29], and two from a third passage blastocyst [29]. There was one pregnancy [26] established from a sixth passage blastocyst [29], but it was lost at about seventy to eighty days. McWhir said that the lack of births from higher passage embryos was due to other factors, such as infections. The claim was disproved in the cell-cycle portion of the experiment.

In the cell-cycle portion, the researchers found no significant difference between variations in concentrations of MPF proteins among the blastocysts whose karyoplasts were in the quiescent state. There were thirty-four embryos from the cell-cycle experiment, which developed from passages six to thirteen karyoplasts and transferred into ewes. The thirty-four embryos produced eight fetuses and resulted in five live births, all exhibiting characteristics of the same female Welsh Mountain lamb. Two of the lambs died within a few minutes of birth and a third one died ten days after birth due to unknown factors. The remaining two lambs, named Megan and Morag, were healthy and normal after nine months and could reproduce normally. Morag became pregnant by a ram [30] when she was eighteen months old and had a lamb of her own. Of the two, Megan lived to at least age ten in 2005.

The results of the second half of the experiment enabled the scientists to show that the quiescent phase is the period of time when chromatin [31], or a mix of DNA and protein, undergoes differentiation [23] and modification resulting in successful blastocysts past the sixth passage. The quiescent phase enables researchers a greater window of time to work with DNA. The window enabled the Roslin team to genetically engineer the sheep [6] Polly, as reported in the 1997 article "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts." The 1995 and 1996 experiments also opened the possibility of cloning [13] a sheep [6] from an already differentiated adult somatic cell [32], which led to the creation of Dolly the Sheep [33] in 1997.

"Sheep Cloned by Nuclear Transfer from a Cultured Cell Line" is a precursor to many other
scientific experiments involving cloning. Campbell and his colleagues showed that differentiated cell’s DNA had the ability to become totipotent. Totipotent cells could create many offspring from cell line, thus producing identical offspring. In the early twenty-first century, embryology and cloning methods use the techniques and theories developed from these experiments.

Sources


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