"Viable Offspring Derived from Fetal and Adult Mammalian Cells" (1997), by Ian Wilmut et al. [1]

By: Bartlett, Zane Keywords: Dolly the sheep [2]

In the 1990s, Ian Wilmut [3], Jim McWhir, and Keith Campbell performed experiments while working at the Roslin Institute in Roslin, Scotland. Wilmut, McWhir, and Campbell collaborated with Angelica Schnieke and Alex J. Kind at PPL Therapeutics in Roslin, a company researching cloning [4] and genetic manipulation for livestock. Their experiments resulted in several sheep [5] being born in July 1996, one of which was a sheep [5] named Dolly born 5 July 1996. Dolly was the first sheep [5] cloned and developed from the nuclei of fully differentiated adult cells, rather than from the nuclei of early embryonic cells. They published their results in "Viable Offspring Derived from Fetal and Adult Mammalian Cells" (abbreviated Viable Offspring) on 27 February 1997.

In 1993, Wilmut, Campbell, and collaborators experimented on cow [6] embryos at the Roslin Institute. These experiments helped develop the theory that differentiated cells in an appropriate environment could develop similar to embryonic cells. For the process of cloning [4] organisms without defects, the cow [6] embryo experiments revealed the optimal cell cycle stage and the need to avoid chromosomal damage when transferring nuclei.

Further information came from experiments described in the 1996 article titled "Sheep Cloned by Nuclear Transfer from a Cultured Cell Line." Campbell, McWhir, Ritchie, and Wilmut conducted experiments in which they cloned sheep [5] from an established embryonic cell line experiments that produced the sheep [5] named Megan and Morag. The scientists developed techniques and concepts from the sheep [5] experiments, including how transferring nuclei during the resting state in the cell cycle called quiescence improves success rates. In the experiments, the scientist removed the nucleus [7] from an egg [8] cell and inserted a donor egg [8] cell to replace the removed nucleus [7], a modified version of the technique called somatic cell nuclear transfer [9].

Wilmut was part of various animal embryo experiments at the Roslin Institute. Schnieke studied bioengineering at the Heinrich-Pette Institute in Hamburg, Germany, and subsequently at the Whitehead Institute [10] of the Massachusetts Institute of Technology [11] in Cambridge, Massachusetts. Before coming to the Roslin Institute, she researched modes to transmit genetic material into cells using RNA viruses of the *Retroviridae* family called retroviral vectors to incorporate therapeutic genes [12] into host cells. She also worked on the production of cross-species genetic modified or transgenic animal models, and preventing specific gene expression in a process called gene knockout [13].

McWhir worked with embryonic stem cells [14] in livestock at the University of Cambridge in Cambridge, United Kingdom, before working at the Roslin Institute. Kind was Schnieke's husband and worked at PPL Therapeutics. Keith Campbell had previously studied the reprogramming of cell nuclei as well as the transplantation of frog [15] nuclear material. Campbell used an experimental method developed by Robert Briggs and Thomas Joseph King in 1952 called nuclear transplantation [16] at the Institute for Cancer Research [17] in Fox Chase, Pennsylvania, which in 1974 became the Fox Chase Cancer Center in Philadelphia, Pennsylvania.

Alan Coleman directed research at the Roslin Institute at the time of the Dolly experiments. Coleman received his PhD in 1974 while studying with John Gurdon [18] at Cambridge University [19], he worked on theories of cloning [4], and he often used frogs for his research. Gurdon's 1975 experiments did not show that adult cell nuclei could create fully developed adult frogs. However, after the sheep [5] experiments in 1996 showed the success of transplanting cells in quiescence, the scientists at the Roslin Institute hypothesized that they could clone a mammal [20] from adult cells, despite Gurdon's results that indicated otherwise. Wilmut later said that Coleman was somewhat skeptical, due to his experience with Gurdon, of what the scientists could accomplish.

The researchers tested their hypothesis with embryonic, fetal, and adult sheep [5] cell samples. The embryonic cells came from a day-nine embryo, the fetal cells came from a day-twenty-six fetus [21], and the adult cells came from a mammary gland of a six-year-old female sheep [5] (ewe) that was in her last trimester [22] of pregnancy [23]. Schnieke suggested using the mammary cells because they appeared similar to the embryo cells used to clone the first sheep [5] Megan and Morag in the 1995 sheep [5] experiment. The scientists used the method derived from 1995 experiments "Sheep Cloned by Nuclear Transfer from a Cultured Cell Line" to implant the cells of different ages into recipient egg [8] cells.

The cells began embryo formation and the scientists then transferred the embryos that developed into a thin-walled early embryo, called a blastocyst [24], into awaiting ewes, and they used ultrasound [25] to check on prenatal development. The results of the ultrasound [25] showed twenty-one fetuses developing normally in fifty to sixty days into the experiment. After the fifty to sixty day interval, the scientists continued to perform ultrasounds in fourteen-day intervals. Sixty-two percent of the fetuses were lost during the intervals after the fifty to sixty days, which, they suggested in the experiment was much greater than the natural prenatal loss of six percent.

The scientists dissected ewes whose fetuses did not survive to investigate why the fetuses did not develop. They found that four of the

dead fetuses were specimens from embryo-derived cells. Two of these fetuses had abnormal liver development, but other than this defect, there was no sign of infection or other irregularity in development from the ewes or the fetuses.

Eight ewes gave birth to live lambs and the offspring represented each of the three cell types, four lambs were born from embryo-derived cells, three were born from fetal cells, and one was born from adult mammary-derived cells. There was one lamb born from fetal cells that died a few minutes after birth. After conducting a post-mortem analysis, the researchers found no abnormalities or signs of infection in this lamb. The researchers concluded that of all of the lambs born, twelve and a half percent died soon after birth. They considered the loss as similar to the eight percent perinatal loss of sheep [5] born naturally. Each of the lambs showed characteristics of the breed that donated the nuclei and not the characteristics of the breed that donated the cytoplasts. These characteristics indicated that the lambs born were clones of the cell that donated their nuclei and not from the originating egg [8] (oocyte [26]) donor. DNA analysis also indicated that each of the lambs' DNA came from their nuclear donors.

The researchers recommended further experimentation to determine exactly what stage in the cell cycle is optimum for deriving nuclear and oocyte [26] donors. The results of their experiment point to using cells that are in the quiescent state, but the experiment itself did not use the optimum cell cycle stage to retrieve donors' oocytes.

In the 1997 article, the scientists reflected on the sheep [5] born from mammary cells, Dolly. According to the scientists, she was the first mammal [20] to develop as a clone from an adult cell. They also said that there is a possibility that among the cell culture there could have been an undifferentiated stem cell present. An undifferentiated stem cell could have been responsible for allowing Dolly to develop to term. Regardless of this small probability, the birth of Dolly showed that once cells mature, control of gene expression is by the cell nucleus [7] as well as changes in the cytoplasm. According to Campbell, this experiment helped to explain the reprogramming of cell DNA and consequently, the process of differentiation [27] of stem cells [28] into specific types of cells.

After the results of the experiment were published, Dolly and the scientists responsible for cloning [4] her received much publicity. Dolly had her photo taken by sixteen film crews and sixty photographers within the first few weeks of the announcement. Wilmut later said that Dolly, amidst all of the attention, only became more tame and spoiled than she was before. Reactions from the media were at first critical and negative. According to Campbell, the negative attitudes toward Dolly and cloning [4] came largely from implications that the same techniques would enable scientists to clone humans [29].

Wilmut later mentioned that Gurdon reported being shocked that Dolly existed because Gurdon could not produce similar results in his own experiments with frogs using adult nuclei. The Vatican and other religious groups voiced fears about cloning [4] humans [29]. The President of the United States in 1997, Bill Clinton, called for a worldwide moratorium on similar experiments and asked a bioethics commission to report on the implications of the Dolly experiment and the ethical questions in relation to human cloning [4]. Some scientists claimed that Dolly was not a clone of an adult cell at all, but that she developed from a stem cell present in the mammary tissue. Another paper published in 1998 that showed Dolly indeed developed from an adult differentiated cell and refuted these claims.

The controversies behind the Dolly experiment diminished with time as other experiments verifyed the results of those at the Roslin Institute. Other scientists soon cloned other livestock, and scientists in Japan cloned offspring from a Japanese Black cattle bull named Kamitakafuku in 1998. The Dolly experiment also enabled a later experiment, "Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts" in which researchers cloned sheep [5] that had human genes [12].

Dolly developed as normal sheep [5] do. Dolly bred with a male sheep [5] and gave birth to six lambs, the first born in 1998. In autumn of 2001, Dolly developed arthritis. The cause of the arthritis was unknown, but it was not attributed to her cloned status. In 2000, one of the cloned sheep [5] in the experiment died of a pulmonary virus that causes incurable tumors in sheep [5]. Dolly died of the same disease on 14 February 2003, likely due to exposure to this virus in 2000.

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