

## Karl Oskar Illmensee (1939–)

Karl Oskar Illmensee studied the cloning and reproduction of fruit flies, mice, and humans in the US and Europe during the twentieth and twenty-first centuries. Illmensee used nuclear transfer techniques (cloning) to create early mouse embryos from adult mouse cells, a technique biologists used in later decades to help explain how embryonic cells function during development. In the early 1980s, Illmensee faced accusations of fraud when others were unable to replicate the results of his experiments with cloned mouse embryos. Illmensee also worked with human embryos, investigating how embryos split to form identical twins.

Illmensee was born in Lindau, Austria, in 1939. At the Ludwig Maximilians Universität in München, Germany, he studied chemistry and biology before beginning his PhD research in the Zoologisches Institut der Universität (University Department of Zoology), part of the Ludwig Maximilians Universität. Illmensee studied the developmental genetics of fruit flies. Two other researchers, Robert Briggs and Thomas King at the American Institute for Cancer Research in Washington, USA, had cloned frogs in the 1950s. Illmensee attempted to use Briggs and King's method to clone fruit flies (*Drosophila melanogaster*).

Despite failing to clone an adult fly, Illmensee published the results of his research in 1968. Illmensee exchanged the nucleus of an unfertilized fly egg with a nucleus from a fertilized fly egg, a process called nuclear transplantation. Of the 118 eggs that had undergone nuclear transplantation, 11 began to develop, and one developed into the larval stage, the stage right before adulthood in fruit flies. Illmensee had not cloned an adult fly, but he had created a clone that survived to the larval stage of development. He completed his PhD in 1972.

In the early 1970s, Illmensee traveled to the Institute for Cancer Research, which later became the Fox Chase Cancer Center, in Philadelphia, Pennsylvania. There, Illmensee worked with Beatrice Mintz to manipulate the unfertilized eggs (oocytes) of mice. Mintz had obtained mouse cancer cells from a malignant teratoma (teratocarcinoma cells). Mintz and Illmensee inserted those teratocarcinoma cells into early stage mouse embryos, called blastocysts. They then implanted those blastocysts into mice, which had been treated with hormones to mimic pregnancy (called pseudopregnancy). The offspring of those mice were called mosaic mice, as they inherited traits from both the cancer cells and normal cells. Offspring of mosaic mice also inherited cancerous cells, though the mice did not develop cancer.

In 1977, Illmensee worked with Peter Hoppe on cloning mice at the Roscoe B. Jackson Memorial Laboratories in Bar Harbor, Maine. Shortly after a mouse sperm fertilizes a mouse egg, the mouse egg contains two nuclei, a maternal pronucleus from the egg, and a paternal pronucleus from the sperm. Each pronucleus contains only half of the genetic material found in the nuclei of normal cells. Together, those pronuclei combine to create the whole genome required for the embryo to develop. In their experiments, Illmensee and Hoppe removed one of the pronuclei from the fertilized mouse egg and placed the egg in a liquid that included ingredients to encourage the pronucleus to divide (cytochlasin B). The single pronucleus remaining in the egg then created a copy of itself, meaning the egg had the correct amount of genetic material required for development. Once those eggs developed into early embryos in the lab, Illmensee transferred them to pseudopregnant female mice. Illmensee and Hoppe reported seven live births out of a total of 135 manipulated eggs. Hoppe and Illmensee called those mice uni-parental, stating that the technique would enable scientists to better control and explain how embryos develop.

In 1978, Illmensee moved to become a professor at the University of Geneva in Geneva, Switzerland. Hoppe visited Illmensee at the University of Geneva during 1979 and 1980. Continuing their collab-

oration, Illmensee and Hoppe began to create hybrid cells, cells that contained genetic material from two different species. Hoppe and Illmensee created rat-mouse hybrid cells and human-mouse hybrid cells. Illmensee and Hoppe injected those hybrid cells into early stage mouse embryos, called blastocysts. They then transferred the blastocysts into pseudopregnant female mice, which gave birth to live mice. Mice created with rat-mouse hybrid cells produced proteins found in rats. Mice created with human-mouse hybrid cells produced proteins found in humans.

In 1981, at the University of Geneva, Illmensee and Hoppe transplanted nuclei from embryonic mouse cells into fertilized mouse eggs that lacked nuclei, a process called nuclear transfer. The eggs developed to the early blastocyst stage in the lab before being transferred to pseudopregnant female mice. Illmensee and Hoppe transplanted 179 developing eggs and recorded three live births. Later, those mice produced offspring that had traits and characteristics of the genes contained in the donated nucleus. Illmensee and Hoppe published their results, and the report attracted worldwide press attention. Following the above work, in 1981, Illmensee received the Marcel Benoist research prize for the most useful scientific discovery in Switzerland or by a Swiss national in the previous year.

In the summer of 1982, Illmensee attempted to use the nuclear transfer technique to transfer the nuclei of mouse teratocarcinoma cells into fertilized mouse eggs with their nuclei removed (enucleated). Three members of Illmensee's laboratory team, Denis Duboule, Joachim Huarte, and Kurt Bürki, attempted to replicate Illmensee's results, but could not get their mouse nucleus and egg transplantation experiments to work. Bürki accused Illmensee of falsifying data at a University of Geneva conference in January of 1983. Bürki provided Marco Crippa, who led the biology department at the university, a report regarding his concerns about Illmensee's research. Crippa and other professors confronted Illmensee in May of 1983, and Illmensee signed a letter admitting that his work had been manipulated inappropriately or not conducted at all. The letter sparked an internal review of Illmensee's work at the University of Geneva, and a further investigation of work Illmensee had done at the Roscoe B. Jackson Memorial Laboratories.

By June 1983, the popular press reported the accusations, and other researchers began to make statements about Illmensee's work. Several researchers admitted they had attempted unsuccessfully to reproduce the results Illmensee and Hoppe had reported in 1981. For example, James McGrath and Davor Solter at the Wistar Institute of Anatomy and Biology in Philadelphia, Pennsylvania, failed to replicate Illmensee and Hoppe's work, reporting their unsuccessful results in 1984. Another group, led by Neal L. First at the University of Wisconsin in Madison, Wisconsin, also failed to reproduce Illmensee and Hoppe's results. An external review of Illmensee's research was organized by the University of Geneva in June 1983, comprised of lawyers and experts in mammalian development. Neither the Roscoe B. Memorial Laboratories nor the University of Geneva internal reviews found definitive evidence of fraud. Likewise, the external review team accepted Illmensee's version of events but conceded that Illmensee kept poor records. The external reviewers recommended that Illmensee repeat his work alongside international collaborators, starting with the transfer of mouse teratocarcinoma nuclei to enucleated fertilized mouse eggs.

In 1988, Illmensee left the University of Geneva and became professor of molecular embryology at the University of Salzburg in Salzburg, Austria. Illmensee worked on repeating the work requested by the external reviewers. In two articles published in 1989 and 1990, Illmensee repeated the required work with Jayek A. Modlinski of the University of Warsaw in Warsaw, Poland, D. Gerhäuser of the University of Geneva, and B. Lioi of the University of Naples in Naples, Italy. None of those experiments resulted in live births. However, Illmensee stated that he had demonstrated the potential of his nuclear transfer techniques.

During the 1990s, Illmensee studied mammalian reproduction, including the biochemistry of human eggs, and the development of organs in the mouse embryo. In 1996, Illmensee became professor of reproductive medicine at the University Hospital of Innsbruck in Innsbruck, Austria.

In 2001, Severino Antinori, who studied medicine at the University of Rome La Sapienza in Rome, Italy, invited Illmensee to a cloning conference in Rome. At the conference, Illmensee met Panayiotis Zavos, who ran an artificial insemination clinic in Lexington, Kentucky. Zavos offered Illmensee a role as director of research for his company, Reprogen. Illmensee accepted Zavos's offer and began

working with Reprogen in 2002. Also in 2002, Illmensee became the Austrian representative for the European Society for Human Reproduction and Embryology Advisory Committee, headquartered in Grimbergen, Belgium.

In the early 2000s, Illmensee published papers about improving artificial reproductive technology (ART) techniques. He discussed a technique called embryo splitting in mice. In the technique, half of the cells from an early embryo were removed and grown in the lab to create two embryos from one. In 2006, Illmensee and Zavos published a paper on somatic cell nuclear transfer (SCNT). SCNT involves removing a nucleus from a cell, and inserting that nucleus into another cell that has had its original nucleus removed. In their work, Illmensee and Zavos cultured cells from an infertile man in the lab. They fused a single adult cell from the infertile man with an egg from the man's wife, and allowed the egg to develop in the lab. Once the egg developed to the four-cell stage, Illmensee and Zavos transferred the early embryo to the uterus of the woman, but the pregnancy did not continue.

Speaking to journalist Sasha Karburg in 2007, Illmensee stated that he attempted to clone humans to help the many infertile couples he had met. Illmensee also said that he found cloning humans particularly difficult. Based on the difficulty he had cloning humans for therapeutic reproduction, Illmensee ended his collaboration with Zavos in May 2007.

At the Genesis Fertility Center in Patras, Greece, Illmensee began working with Mike Levanduski. They also worked at American Fertility Services in New York City, New York. Illmensee and Levanduski studied embryo splitting, a process that can occur in pregnant women and results in identical twins. Levanduski and Illmensee caused human embryos to split in the laboratory, reporting their results in 2010. As the split human embryos progressed to a further stage of development, Levanduski and Illmensee suggested that embryo splitting might be useful for artificial reproductive technologies (ART).

By 2017, Illmensee continued to work at the Genesis Fertility Center in Patras, Greece, continuing his research on ART methods.

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