

Ernest John Christopher Polge (1926-2006)

Twentieth-century researcher Ernest John Christopher Polge studied the reproductive processes of livestock and determined a method to successfully freeze, thaw, and utilize viable sperm cells to produce offspring in animals. In 1949, Polge identified glycerol as a cryoprotectant, or a medium that enables cells to freeze without damaging their cellular components or functions. Several years later, Polge used glycerol in a freezing process called vitrification, which enabled him to freeze poultry sperm, thaw that sperm, and use it to fertilize vertebrate embryos. He later adapted those methods to be applied to several other species including goats, cows, and pigs, which enabled farmers to fertilize livestock with sperm or embryos after long-term storage. Additionally, Polge's development of methods to freeze and store living samples has equipped reproductive health researchers and medical professionals with the abilities to collect and store human sperm.

Polge, commonly called Chris Polge, was born on 16 August 1926 in Buckinghamshire, England in the village of Beaconsfield. His mother, Joan Thorne, was a Quaker at Woodbrooke College in Birmingham, England. His father, Ernest Polge, was a disabled World War I veteran who owned a poultry farm. The second of four children, Polge lived with his family on the poultry farm and attended weekly worship. As the farm grew, Polge's father's physical health declined, so Polge assisted his father with duties around the farm throughout his childhood. At the age of nine, Polge began his secondary education at Gayhurst School in the neighboring village of Gerrard's Cross in Buckinghamshire, England. Polge went on to Bootham School in York, England, on a scholarship, where he played on three different sports teams, as well as participated in debate, theater, and choir. He was a prefect at his school for two years and then was appointed head boy in his final year.

In 1944, Polge was admitted into Reading University in Berkshire, England, where he pursued a degree in agriculture. As he was pursuing a degree of national importance, determined by regulations of the British National Service, Polge was not subject to the draft that began during World War II. He entered an accelerated wartime schooling program, and earned an Ordinary degree in agriculture in two years. In Britain, Ordinary degrees are the equivalent of a bachelor's degree without honors. Following his graduation in 1946, he returned home to work on the farm for six months before accepting a job monitoring farm production costs in Southwest England with the Agricultural Economics Department of Bristol University in Bristol, England.

Shortly thereafter, Polge connected with researcher Alan Sterling Parkes, who headed the Division of Experimental Biology at the National Institute for Medical Research or NIMR in London, England, a government-funded research institute. Parkes researched reproductive biology, specifically the use of hormones to control mammalian reproductive processes. Polge assumed a permanent position as a staff member at NIMR. At the NIMR, Polge began studying the methods of freezing poultry sperm as a doctoral student alongside his supervisor, Audrey Smith. Smith was a medical researcher and clinical pathologist who joined the NIMR to continue her research after serving as a doctor in several hospitals during World War II. She studied neurotransmitter function in marine animals, as well as various diseases she encountered when she practiced medicine.

While working with Smith at the NIMR in the late 1940s, Polge determined one of the first protocols for successfully freezing viable poultry sperm. Polge and Smith conducted multiple experiments regarding the freezing of poultry sperm cells in fructose solutions of varying concentrations. Other researchers had shown in previous experiment that fructose, a kind of sugar, preserves sperm motility, or the ability to physically move. Polge showed that sperm suspended in a fructose solution could survive in temperatures as cold as -79°C , which is a temperature far lower than the freezing

point of water. Preserving the motility of the sperm enabled the sperm to locate, penetrate, and enter the egg, without which fertilization could not occur. Post-thaw, however, Polge found that the sperm did not fertilize eggs. Polge concluded that, although the sperm cells retained their motility after thawing, the sperm was somehow damaged during the freezing process in a way that ruined the sperm's ability to fertilize an egg, and that the right concentration of fructose in the medium had to be found to preserve sperm function as well as motility.

According to Polge's own written account, after several months' hiatus from the research that he had begun in 1947, he returned to his work using an old bottle of fructose medium left over from previous experiments. Unbeknownst to Polge at the time, someone switched out the fructose medium for a bottle of Meyer's egg albumen, which Polge described as a mixture of egg whites and glycerol, another kind of sugar that workers at the NIMR typically used to fix samples to microscope slides. However, Polge discovered that the new solution preserved the full function of the sperm cells, and in 1949 the first chicks fertilized with frozen sperm hatched. Through further research, Polge and Smith confirmed that high concentrations of glycerol were necessary to preserve the function of sperm. Polge's protective medium, otherwise known as a cryoprotectant, regulated the salt concentration when water moved out of the cells. Exposure to high concentrations of salt can damage vital components of the cell, and mediums that regulate salt levels during the freezing process prevent that lethal damage. In 1949, Polge and Smith published their findings on glycerol's effects on frozen sperm cells in the *Nature* article "Revival of Spermatozoa after Vitrification and Dehydration at Low Temperatures." Their research also served as the basis for Polge's doctoral thesis, which he obtained six years later from the University of London in London, England.

After successfully using glycerol to preserve the sperm of poultry in 1949, Polge began working on freezing the sperm of other species. According to Hunter, Polge knew that the use of frozen sperm among livestock breeders held potential for commercial gain in the agricultural market. In the 1940s, farmers and ranchers were limited in their use of sperm to artificially inseminate cows. Bull semen had to be kept refrigerated at 5°C after collection, and its quality degraded even after short-term storage. Polge accordingly adapted his freezing process for bull sperm under Parkes's supervision at the NIMR.

In 1950, Polge and veterinary surgeon Lionel Edward Aston Rowson collaborated to research the storage and thawing of viable bovine sperm. The team worked primarily out of the AI Centre, an artificial insemination center based in Cambridge, England and funded by the British government. Polge was also given a van by the NIMR to use as a mobile laboratory when he visited farms to work on site. Polge and Rowson used glycerol as a cryoprotectant in their experiments, but the freezing process had to be altered to allow for the increased susceptibility of bull sperm to sudden changes in temperature. In 1952, Polge presented their collaborative findings at the Second International Congress on Animal Reproduction in Copenhagen, Denmark, where he showcased the effective technique for fertilization of bovine using frozen sperm. Polge and Rowson's method enabled the commercial cattle industry to inseminate cattle using frozen sperm. Such a technique enabled farmers to optimize their herds by selecting sperm from preferred bulls without the time restrictions of unfrozen sperm, which quickly lost viability. In 1953, Polge went on an international tour to demonstrate the technique to farmers around the world.

Later that year in 1953, Polge returned to the lab at the NIMR and continued studying the effects of glycerol as a cryoprotectant along with Smith and colleague James Lovelock. Smith determined that red blood cells were also protected by glycerol during the freezing process. Lovelock and Polge tested and recorded the effects of using glycerol to protect the sperm of rabbits, bulls, fowls, and herrings. They observed that all sperm samples retained full motility after freezing with glycerol, and proved that the glycerol medium safely preserved sperm from all four species. This indicated that sperm cells from rabbits, bulls, fowl and herrings were all permeable to glycerol, as well.

In 1954, Polge left his position with the Medical Research Council to work at the nearby Unit of Animal Reproduction under the Agricultural Research Council. The Unit of Animal Reproduction was headed by John Hammond and operated out of the Animal Research Station, a research facility located in Cambridge, England. That same year Polge married Olive Kitson, whom Polge had met when she worked as the secretary for the Department of Biophysics and Optics at the NIMR. At his new post with the Unit of Animal Reproduction, Polge continued his animal research. In col-

laboration with Rowson, Polge studied methods of non-surgical embryo transfer in cattle, but their attempts proved unsuccessful. By the late 1950s, Polge focused his work onto a herd of pigs kept on site at the Animal Research Station.

In the early 1960s, Polge collaborated with Philip Dziuk, an associate professor of reproductive physiology on sabbatical leave from the University of Illinois in Champaign, Illinois. In 1961, Polge and Dziuk identified patterns in the dispersal of transplanted pig embryos in a sow's uterus. Along with surgical assistance from Rowson, the team transferred embryos of known colorations for fetal skin pigment into the oviducts, or the tubes that descend from the ovaries, of an unmated sow. They placed embryos that would yield pigs with black coloring in one of the sow's two oviducts and placed embryos that would yield pigs with white coloring in the other. After ninety days of gestation, Polge and Dziuk dissected the sow and examined the developing fetuses. They determined that eggs migrated in the uterus between the sow's two oviducts in random patterns.

In 1962, after Dziuk returned to the US, Polge took on his first PhD student from the School of Agriculture in Cambridge. For several years, Polge and his lab continued to study fertilization and estrus cycles of mature sows in detail. In 1964, Polge conducted trials for a compound that could link together sow estrus cycles as a method of batch breeding. Though the compound was an initial success and was used commercially, it was removed from western European and North American markets a short time later when harmful effects were observed in pregnant sows that consumed it. In 1966, Polge took a year-long sabbatical to the United States, where he served as a consultant for several institutions including the US Department of Agriculture in Washington, D.C., and the National Institutes of Health in Rockville, Maryland.

In the early 1970s, Polge resumed his work with doctoral student Ian Wilmut, who developed one of the first successful methods for freezing and thawing viable boar sperm. After observing the effects of freezing on pig, sheep, and cow embryos in liquid nitrogen, the pair determined that cow morulae, or the ball of cells resulting from early embryo division, and blastocysts or the hollow ball of cells that forms from the morula, remain viable at temperatures as low as 0°C. After tweaking their methods, Polge and Wilmut adapted their freezing process to bovine embryos. That process, when applied, resulted in the 1973 birth of the first calf from a frozen-thawed embryo, Frosty. That alternative method of insemination enabled cattle breeders to select both sets of parental genetic input in livestock offspring, which helped improve livestock quality in the overall market.

The majority of research at Polge's lab throughout the 1970s was conducted by incoming doctoral students and visiting scientists, who Polge mentored. Such research included the micromanipulation of embryos by Neil Moore, a researcher from the University of Sydney in Sydney, Australia. Moore's micromanipulation involved separating early pig and rabbit blastomeres, which are 2-cell or 4-cell embryos, into single embryonic cells to grow more individual embryos. Polge's lab also hosted Steen Willadsen, a postdoctoral fellow from Copenhagen, Denmark. According to Hunter, Willadsen produced a goat-sheep hybrid which was featured on the cover of Nature magazine, and earned Polge's lab attention around the world. Polge also collaborated with Winsten Cheng, a research student from Taiwan, to fertilize pig eggs in vitro, but they never published a paper on the experiment.

In 1979, Polge was elected the director of the Unit of Animal Reproduction, which had been renamed the Unit of Reproductive Physiology and Biochemistry in Cambridge, England. At that time, Polge also served as the chairman for both the Society for the Study of Fertility and the Society for Low Temperature Biology, and he contributed to the editorial boards of several reproductive journals. After eight years, Polge retired from his role as director of the Unit of Reproductive Physiology and Biochemistry and founded his own company, Animal Biotechnology Cambridge, Ltd with partner and geneticist Martin Evans. Their company, located in Cambridge, England, focused on the in vitro production and storage of bull sperm at a commercial level. Animal Biotechnology Cambridge developed methods of sperm separation and studied the production of transgenic animals. However, according to Hunter, Polge found running a business stressful and closed the company in 1996.

In the 1980s, Polge experienced ailing health, but he continued to contribute to the fields of agriculture and cryobiology. Polge published over 160 papers throughout his career. He was elected a member of the Royal Society in 1983, a Fellow of Wolfson College in 1984, and an Honorary Pro-

fessor in the Veterinary School at University of Cambridge in 1989. The US National Academy of Sciences recognized him as a Foreign Associate, Israel awarded him the Wolf Foundation Prize in Agriculture, and he was deemed a Commander of the British Empire in 1992. He also won the eighth Japan Prize for Science & Technology in 1992.

Polge died at home on 17 August 2006, the day after his eightieth birthday.

Sources

1. Blaxter, Kenneth and Noel Robertson. *From Dearth to Plenty: The modern revolution in food production*. Cambridge: Cambridge University Press, 1995.
2. Bradley, Allan, Martin Evans, Matthew H. Kaufman, and Elizabeth Robertson. "Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines." *Nature* 309 (1984): 255-6.
3. "Christopher Polge." *The Telegraph*, September 11, 2006, Obituaries, online. <http://www.telegraph.co.uk/news/obituaries/1528529/Christopher-Polge.html> (Accessed October 23, 2017).
4. Dziuk, Philip J., Christopher Polge and Lionel Edward Rowson. "Intra-Uterine Migration and Mixing of Embryos in Swine Following Egg Transfer." *Journal of Animal Science* 23 (1964): 37-42.
5. Hunter, Ronald H. F. "Ernest John Christopher Polge. 16 August 1926-17 August 2006." *Biographical Memoirs of Fellows of the Royal Society* 54 (2008):275-96.
6. Johnson, Lawrence A. "In memoriam: Ernest John Christopher Polge (1926-2006)." *Theriogenology* 70 (2008): 1172-3.
7. Lovelock, James E. and Christopher Polge. "The Immobilization of Spermatozoa by Freezing and Thawing and the Protective Action of Glycerol." *Biochemical Journal* 58 (1954): 618-22. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1269950/pdf/biochemj01078-0108.pdf> (Accessed July 13, 2017).
8. Polge, Christopher, Audrey Ursuka Smith, and Alan Sterling Parkes. "Revival of Spermatozoa after Vitrification and Dehydration at Low Temperatures." *Nature* 164 (1949): 666-70.
9. Polge, Christopher. "Lionel Edward Aston Rowson, O. B. E. 28 May 1914-26 July 1989." *Biographical Memoirs of Fellows of the Royal Society* 46 (2000): 484-97.
10. Polge, Christopher. "The work of the Animal Research Station, Cambridge." *Studies in History and Philosophy of Biological and Biomedical Sciences*. 38 (2007): 511-20. <http://europepmc.org/abstract/med/17543843> (Accessed October 23, 2017)
11. Polge, Christopher, Ian Wilmut, and Lionel Edward Rowson. "The low temperature preservation of cow, sheep and pig embryos." *Cyriobiology* 11 (1974): 560.
12. Schnieke, Angelika E., Alexander J. Kind, William A. Ritchie, Karen Mycock, Angela R. Scott, Marjorie Ritchie, Ian Wilmut, Alan Colman, and Keith HS Campbell. "Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts." *Science* 278 (1997): 2130-3.
13. Willadsen, Steen M. "Nuclear transplantation in sheep embryos." *Nature* 320 (1986): 63-5. <http://europepmc.org/abstract/med/3951549> (Accessed October 23, 2017).