

# Christopher Polge and Lionel Edward Aston Rowson's Experiments on the Freezing of Bull Spermatozoa (1950-1952)

In 1952, researchers Christopher Polge and Lionel Edward Aston Rowson, who worked at the Animal Research Center in Cambridge, England, detailed several experiments on protocols for freezing bull semen for use in the artificial insemination of cows. Freezing sperm extends the life of a viable sperm sample and allows it to be used at later times, such as in artificial insemination. The researchers examined the effects of freezing conditions on bull sperm and how well they produce fertilized embryos once thawed. Polge and Rowson concluded that bull sperm can retain its fertility throughout the freezing process and that frozen bull sperm can yield pregnancy rates of up to seventy-nine percent. Polge and Rowson provided the first conclusive evidence that frozen mammalian sperm, once thawed, can produce viable pregnancies.

In the early 1900s cryobiology, or the study of freezing living cells and tissues, was an emerging field of science. Freezing cells allows them to be stored at low temperatures for extended periods, well beyond the normal lifespan of cells in a human body or culture. Those cells can then be thawed and used at a desired time. However, the freezing process can damage cells beyond repair through the formation of ice crystals in the interior of cells, or by trapping high concentrations of salt in the cells when water is drawn out of them. By 1950, researchers had not successfully frozen animal cells or tissues without killing them during the process.

Polge was one of the earliest researchers in the field of cryobiology, and he worked at the University of Cambridge in Cambridge, London, within the National Institute for Medical Research in London, England. He and two of his colleagues initially discovered the potential use of glycerol as a cryoprotectant, or a chemical medium that helps preserve the function of live cells under freezing conditions. Rowson worked at Animal Research Center in Cambridge, England and was one of the first veterinarians to practice artificial insemination and embryo transplants in livestock. Rowson collaborated with Polge on several experiments that involved the reproductive practices of livestock throughout the mid 1900s.

In 1949, Polge and a small team of researchers determined that glycerol, when mixed with cultures of live sperm cells, preserved the function of sperm under freezing conditions for a variety of vertebrate animals. Glycerol is a sugar-based alcohol that is naturally found in cells. After several subsequent experiments, researchers found that fowl, or bird, sperm retained the greatest motility after being thawed from a frozen state and could produce viable embryos when used in fertilization afterwards. Motility of sperm refers to the physical movement of a sperm cell in its environment, as sperm need to move through their environment in order to find and fertilize eggs. Higher motility typically improves the chances of the sperm cell finding and penetrating an egg. Thus, motility is a factor in determining the potency, or effectiveness, of a sperm sample. However, according to Polge and Rowson, mammalian sperm reacted differently than fowl sperm under the same conditions. Researchers tested rabbit, guinea pig, goat, horse, and bull sperm with similar freezing protocol as those used with fowl sperm. All mammalian samples showed lower rates of sperm cell revival upon thawing. According to Polge and Rowson had been unsuccessful in using frozen mammalian sperm for later fertilization efforts.

In 1950, Polge and Rowson began a series of two experiments in which they attempted to freeze mammalian sperm that can be used to successfully fertilize eggs. In their first experiment, the researchers examined the effect of adding glycerol into bull semen samples. Polge and Rowson

divided a sample of bull semen into three separate parts. They exposed the three parts to different conditions, and then tested the potency of each sample. Polge and Rowson diluted the first of the samples with an equal volume of a standard material known as Wellcome citrate buffer containing fifty percent egg yolk at a temperature of 30 degrees Celsius. The researchers stored that sample for twenty-four hours in a refrigerator at 5 degrees Celsius. The first sample served as a control in the experiment, to show whether or not the sperm sample was viable after twenty-four hours without the addition of glycerol. The researchers then diluted the second of the samples with an equal volume of the same yolk-buffer mixture, but they added thirty percent glycerol to the buffer. They stored that sample for twenty-four hours in a refrigerator at 5 degrees Celsius. That sample would demonstrate the effect of adding glycerol to a sperm sample, while maintained in the same environment as the untreated sperm sample. Polge and Rowson then diluted the third of the semen samples using the same yolk-buffer-glycerol mixture as the second sample. That third sample, however, was slowly cooled from 30 degrees Celsius to -79 degrees Celsius. The researchers then thawed the third sample to 40 degrees Celsius, or roughly the body temperature of a healthy cow. That sample would demonstrate the effect of adding glycerol into a sperm sample, and then subjecting that mixture to freezing conditions temporarily.

After a twenty-four-hour incubation period, Polge and Rowson used sperm from each of the three samples to artificially inseminate a total of sixty cows that the researchers checked for signs of pregnancy six weeks later. According to the researchers, the first sample of sperm successfully fertilized ten cows out of twenty inseminated for a fertilization rate of fifty percent. Those results indicated that the untreated sperm sample kept at cool temperatures produced a pregnancy in nearly half of the insemination attempts it was used in. The second sample yielded a fertilization rate of seventy-six percent, which indicated that sperm mixed with glycerol and stored at cool temperatures resulted in pregnancies in over three quarters of the insemination attempts it was used in. The third sample, however, failed to fertilize any cows. That indicated that sperm mixed with glycerol and then frozen did not result in any pregnancies when used in an insemination attempt.

In their first experiment, Polge and Rowson observed that the addition of glycerol did not impair the function of sperm when added to a semen sample. The researchers observed that the bull sperm frozen with glycerol resulted in no pregnancies and noted that the bull sperm chilled with glycerol not only yielded viable embryos, but actually produced more pregnancies than the untreated sperm sample did. Polge and Rowson concluded that the addition of glycerol was not responsible for inhibiting fertilization by the bull sperm. Some other factor had damaged the frozen sperm. The researchers theorized that the presence of glycerol could potentially improve the fertilization rates of bull sperm, but the limited sample size of the experiment prevented the authors from definitively concluding anything in that regard. However, Polge and Rowson proved that glycerol, already known as an effective cryoprotectant for fowl sperm cells, could be safely added to samples of bull semen while still preserving sperm function.

In their second experiment, which occurred in 1951, Polge and Rowson determined the best method for adding glycerol into the bull sperm in a way that yields sperm that can successfully fertilize an egg, even after the sperm had been exposed to freezing conditions. The authors claimed that the premise of their second experiment was inspired by something they had observed during their earlier work. Polge and Rowson noted that they had previously concluded that equilibrating the glycerol with the sperm samples for several hours prior to freezing improved revival rates of the sperm post-thaw. Equilibration refers to the process of allowing two components of a mixture to combine evenly within it.

Employing those methods, Polge and Rowson conducted a second experiment in which they developed a new freezing technique for the bull semen. The researchers attempted to reproduce the boosted sperm revival rate through the equilibration of the glycerol with the sperm prior to freezing. Immediately after collecting the semen samples, the authors diluted them with an equal volume of citrate buffer at 28 degrees Celsius. The dilution was performed immediately after collection, according to the authors, in order to prevent any degradation to the sperm between the time of collection and the time of experimental treatment. Polge and Rowson then cooled those samples to 5 degrees Celsius over a four-hour period in order to chill the sperm for short-term storage. Once cooled, they then diluted the samples again with an equal volume of the citrate buffer, containing

twenty percent glycerol, and left the samples to sit overnight at 5 degrees Celsius. That gradually introduced the glycerol into the mixture of the sperm cells, and allowed the glycerol to thoroughly mix with the sperm cells during the longer incubation period.

The next day, the researchers gradually cooled the samples from 5 degrees Celsius, a chilled storage temperature, to -79 degrees Celsius, a freezing temperature. They then stored those frozen samples in sealed containers for various periods of time, ranging from two hours to eight days. Upon thawing each of those samples, the researchers immediately artificially inseminated cows with the previously frozen semen. The researchers performed the procedure on thirty-eight cows in total. After six weeks, Polge and Rowson observed that thirty of those cows yielded viable pregnancies, resulting in a fertilization rate of seventy-nine percent. That showed an improvement when compared to the results of using the frozen glycerol-sperm sample in the first experiment, which had yielded a zero percent fertilization rate when used to inseminate cows without equilibrating first. Polge and Rowson concluded that bull sperm can retain its capacity for fertilization throughout the freezing process when following the protocol they detailed in the experiment. The researchers noted that treating bull sperm with a glycerol medium could improve fertilization rates when used in insemination, as was first seen in their 1950 experiment. However, they also noted that their current data was insufficient to conclusively prove it.

After conducting those two experiments, Polge and Rowson discussed the impacts of their findings. They argued that their work offered insight into the permeability of cells, or the natural tendency of cells to take in the chemicals of their cellular environment, as well as the behavior of cells at extremely low temperatures. The researchers also noted that their proposed technique for freezing bull semen could allow those samples to be stored for longer periods of time than contemporary practices allowed. Those sperm cells, once thawed, could retain and even have an improved capacity to fertilize cows. Lastly, Polge and Rowson conceded that more work was needed to further increase the length of time that the samples could be stored for under freezing conditions.

Polge and Rowson's experiments developed a method for freezing bull semen in a way that yielded viable pregnancies in cows after artificial insemination. As of 2017, freezing semen of desirable studs is a common practice in modern day agriculture. It is also prominently used in the context of animal breeding. Polge and Rowson's experiments also contributed to the research and development for freezing and storage protocol of other mammalian sex cells, including humans.

## Sources

1. 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.
2. 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).
3. 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. <http://rsbm.royalsocietypublishing.org/content/roybiogmem/46/483.full.pdf> (Accessed July 13, 2017).
4. Polge, Christopher, Audrey Ursuka Smith, and Alan Sterling Parkes. "Revival of Spermatozoa after Vitrification and Dehydration at Low Temperatures." *Nature* 164 (1949): 666-670.
5. Polge, Christopher and Lionel Edward Aston Rowson. "Fertilizing Capacity of Bull Spermatozoa after Freezing at -79°C." *Nature* 169 (1952): 626-7.