“Revival of Spermatozoa after Dehydration and Vitrification at Low Temperatures” (1949), by Christopher Polge, Audrey Ursula Smith, and Alan Sterling Parkes

In the 1949 article “Revival of Spermatozoa after Dehydration and Vitrification at Low Temperatures,” researchers Christopher Polge, Audrey Ursula Smith, and Alan Sterling Parkes demonstrated that glycerol prevents cells from dying while being frozen. Polge and his colleagues discussed several procedures in which they had treated sperm cells from various species with glycerol, froze those cells, and then observed the physiological effects that freezing had on the treated sperm. The researchers concluded that glycerol safely preserves sperm samples from a variety of species. Polge, Smith, and Parkes’s 1949 article detailed one of the first successful uses of a chemical medium to preserve viable cells in a frozen state, a process that eventually enabled the first vertebrate embryo to be successfully conceived using frozen sperm.

A team of three authors wrote the article “Revival of Spermatozoa after Dehydration and Vitrification at Low Temperatures.” In the late 1940s, Polge and Smith collaborated with their supervisor, Parkes, to research the effects of freezing conditions on sperm samples from a variety of species. All three scientists worked at the National Institute for Medical Research in London, England, an organization funded by the British government’s Medical Research Council, also based out of London.

Throughout the 1940s, researchers worked to determine a process for safely freezing cells that would be viable for use after thawing. Freezing a sperm sample enables that sperm to fertilize an embryo at a specific time after it was initially produced by a male, but freezing can have lethal or disabling effects for the cell. Under natural conditions, exposing cells to freezing temperatures results in total cell death. Formation of ice crystals within and around the cell ruptures the membrane of the cell, resulting in cell death. High salt concentrations within the cell that result from water leaving the cell can also irreparably damage cellular components. By 1949, no frozen sperm cell of any species had successfully fertilized an egg and resulted in a viable embryo.

In “Revival of Spermatozoa,” Polge, Smith, and Parkes report how they tested multiple freezing protocols on samples of poultry sperm in an effort to produce thawed sperm cells that could ultimately yield viable embryos through fertilization. Although the authors did not divide the one-page article into sections, there are four themes or sections that exist within the article. In the first section, the authors introduce prior experiments in which researchers had frozen and studied sperm cells. In the next section Polge, Smith, and Parkes detail their own experiments, in which they observed the effects that mixing glycerol and similar compounds with human sperm, rabbit sperm, and fowl sperm have on the functionality of the different sperm types after being frozen and thawed. In the third section, the authors discuss results when applying similar techniques to freeze dried sperm samples. In the fourth section, the authors summarize the implications of their research and allude to further research.

In the first section, Polge, Smith and Parkes summarize earlier research that involved the freezing of sperm cells through a process called vitrification. Vitrification is the process by which materials, such as cells, are frozen in a manner that prevents the formation of ice crystals. The authors note that human sperm is resistant to the vitrification process, but they do not explain that claim. They also state that when human sperm samples are frozen in bulk and subsequently thawed, the sperm

samples move better than smaller sperm samples that undergo the same process. The authors have no explanation for that phenomenon, but they hypothesize that effects on the surface of the sperm cells have a greater impact on the viability of those samples than the process of the freezing itself.

Continuing the first section, the authors explain that higher viability rates have occurred in frozen samples of frogs and fowl sperm, particularly when sugar solutions are incorporated into the samples prior to freezing. The authors mention that the sugar solutions dehydrate the samples, but do not specify the exact composition of the solutions.

In the next section, Polge, Smith and Parkes examine the effects of glycerol treatment on various sperm samples. The authors first discuss their motivation for testing glycerol as a cryoprotectant, which is a solution that protects cells during the freezing process. They note that they were motivated by a chance observation, but don't describe that in any further detail. Similarly, Polge, Smith, and Parkes do not outline the methods by which they test the effects of using glycerol on human sperm. Instead, they summarize the results. The authors report that human sperm samples containing glycerol showed higher rates of viability after being frozen and thawed again than the control samples. That suggests that glycerol protects human sperm cells during the freezing process. They also note that a five percent concentration of equal parts semen and ten percent glycerol solution in Baker's fluid was optimal for viability. However, the authors did not define the composition of Baker's fluid. The authors observe that repeated experiments with propylene glycol, which is a compound similar to glycerol, and ethylene glycol, which is another compound similar to glycerol, produced similar results. This suggests that substances with a similar composition to glycerol can also be used as cryoprotectants in freezing mediums for sperm cells.

In the next paragraph, Polge, Smith, and Parkes detail their results of testing the effects of glycerol on rabbit sperm. The authors state that they froze the sperm via bulk vitrification, the exact definition of which is not specified in the article. The authors report that no rabbit sperm regained motility, or the ability to move, after thawing from that process. Polge, Smith and Parkes also note poor results with the use of glycerol in the semen, although they do not provide exact data. The authors suggest that rabbit sperm is susceptible to the toxic effects of glycerol, which they do not define in their paper. The authors also state that a lower five percent concentration of glycerol yielded slightly improved motility in the thawed rabbit sperm, but compared it unfavorably to the results produced by the earlier human samples.

The authors write in the next paragraph that poultry sperm had one of the highest success rates in the experiment. The authors claim that incorporating a forty percent concentration of glycerol and Ringer's solution, which is a salt solution, into the semen sample prior to vitrification revived nearly the entire sperm sample to full motility upon thawing. The article states that the sperm was frozen over a twenty-minute period to temperatures of -79°C, and were kept frozen for up to ten weeks. Those findings provided evidence that glycerol served to preserve sperm cell structure and function even under extremely cold conditions for prolonged periods of time. Polge, Smith, and Parkes also claim that a final concentration of glycerol below ten percent decreased glycerol's preservative effect, while a final glycerol concentration above twenty percent partially immobilized the sperm cells without freezing. Those results implied that the amount of glycerol added into the solution, relative to the sperm sample, is crucial to the success of glycerol's preservative effect.

In the third section, Polge, Smith, and Parkes discuss the use of glycerol as an approach to freeze drying sperm cells. Freeze drying entails combined freezing and removal of nearly all of the water from the sample cells, although this process is not identified in the article. The authors explain that they vitrified equal parts of poultry semen and Ringer's solution containing twenty percent glycerol to -79ºC in a flask. According to the authors, that mixture was then dried for three hours under a high-vacuum distillation system, and then reconstituted with a volume of water equal to that collected during the distillation. Upon warming the sample to 40ºC, the authors report that up to half of the sperm cells in the sample recovered motility. The authors write that they repeated the process eight times, all of which yielded consistent results. Those results demonstrated that freezing and the subsequent removal of over ninety percent of the sperm cells' water resulted in no disabling side effects with regard to cell structure and function. Those results also imply that glycerol's preservative effects apply to the freeze-drying process, as well. However, Polge, Smith, and Parkes note that leaving glycerol in the proximity of the cells for more than two hours at room

temperature resulted in cell death due to toxic effects. The authors state that they did not test the fertilizing capacity of the sperm cells after thawing and rehydration.

In the final section of the article, Polge, Smith, and Parkes summarize the results of the freeze-drying experiment. They theorize that high proportions of water can be dehydrated from fowl sperm cells without permanent damage to the cells. The authors state that the extent of glycerol's influence on those results was unclear. They conclude that similar studies with sperm from other species are in progress, although they do not specify whether or not they are the researchers conducting those studies.

Polge, Smith, and Parkes's article continues to be cited into the twenty-first century regarding matters of the freezing and storage of sperm cells, nearly fifty years after its original publication. The article has been academically and professionally cited over 1800 times, including a reference in the World Health Organization's laboratory manual for the handling of human sperm. The article is also cited by works on a variety of topics including the examination of samples under microscopes at freezing temperatures, methods of storage of tissue cultures, and the use of cryoprotectants in the freezing and storage of other live microorganisms.

Sources

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