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The history of cryotechnologies in reproductive medicine: From randomness to stability

Abstract. *The article provides an overview of the historical milestones in cryobiology, a scientific field that has found widespread practical application in reproductive medicine. Cryotechnologies have revolutionized assisted reproductive technologies, offering invaluable tools for the storage, protection, and management of reproductive cells such as sperm, oocytes, and embryos. The technology began with the first successful attempts at sperm cryopreservation, which not only demonstrated the possibility of preserving male gametes but also inspired a wave of research aimed at developing cryopreservation protocols for more sensitive and complex biological entities, including oocytes and preimplantation embryos. Cryopreservation has become a crucial component of fertility preservation, offering new opportunities for*

individuals and couples facing medical treatments that could compromise their reproductive potential, as well as providing options for delaying parenthood. Given the critical role of cryotechnologies in modern reproductive medicine, this article delves into the historical context of these developments, exploring the key breakthroughs that have shaped this field. The authors conducted an in-depth analysis of existing literature, drawing on a wide range of scientific databases to highlight the global impact of cryobiology on the current successes in reproductive medicine. Furthermore, the article presents the results of the authors' own research and practical experience in the field of reproductive cryobiology, with a particular focus on the application of these technologies in Ukraine. The review underscores the challenges and opportunities that have emerged throughout the history of cryopreservation, as well as ongoing efforts to improve and optimize these methods to further enhance infertility treatment outcomes. The discussion also addresses ethical and logistical considerations related to cryopreservation, particularly in the context of long-term storage and future use of cryopreserved materials. As cryobiology continues to evolve, its integration into reproductive medicine will undoubtedly lead to further innovations, making it a cornerstone of infertility treatment and reproductive health worldwide.

Keywords: *cryopreservation; vitrification; slow freezing; thawing; in vitro fertilization; assisted reproductive technologies*

Introduction.

Over the past decades, we have witnessed a paradigm shift in the use of assisted reproductive technologies (ART), driven by the convergence of technological advancements, optimization of patient preparation protocols, and an increasing demand for infertility treatment services (Petrushko, Piniaiev & Yurchuk, 2021). Concurrently, there has been a development in cryotechnologies: from experimental stages to their modern innovative applications in reproductive medicine, which stands as a testament to the scientific ingenuity and perseverance of hundreds of talented cryobiologists and cryomedics. By offering innovative solutions for fertility preservation, cryotechnologies have profoundly transformed the field of reproductive medicine, elevating its efficiency to a higher level.

Cryotechnologies play a key role in preimplantation genetic diagnosis and preimplantation genetic screening, as cryopreservation enables genetic analysis and the subsequent selection of a healthy embryo for transfer into the uterine cavity (Giuliano, Maione, Vallefuoco, Sorrentino, & Zuccarello, 2023).

Thanks to donor gamete banks, unique micromanipulations are possible: the transfer of the second polar body, the oocyte nucleus, or the pronuclei of zygotes into a donor oocyte, which is crucial for therapies aimed at replacing defective mitochondria (Smeets, Sallevelt, & Herbert, 2023).

Future technologies in the field of genome editing are impossible without the use of cryotechnologies (Zhu, 2022).

Low-temperature storage ensures the long-term viability and integrity of biological material, which is important for the success of advanced biomedical technologies, contributes to improved patient outcomes, and advances assisted

reproductive technologies.

But the future is impossible without the present. And the present is based on past experience.

The aim of this work is to explore the historical stages of the development of cryotechnologies in reproductive medicine, their impact on current practices, and future prospects in the context of infertility treatment and fertility preservation.

The main objectives of the study include:

1. Analyzing key historical events and discoveries in the field of cryopreservation of reproductive cells and tissues.
2. Examining the evolution of cryopreservation technologies for gametes and embryos.
3. Assessing the impact of the latest cryotechnologies on the development of modern infertility treatment methods.
4. Identifying the main challenges and prospects for the advancement of cryotechnologies in reproductive medicine.

Research methods.

The methodology of the research is based on literature search in scientific databases (Scopus, Web of Science, Pubmed, Google Scholar, MedLine, Embase, Cochrane Library, ScienceDirect) about chronology and milestones of cryobilogical techniques for reproductive medicine.

Results and Discussions.

Sperm Cryopreservation.

The first mention of reproductive cells storing at low temperatures was recorded in 1776. At that time, an Italian cleric and scientist, L. Spallanzani (1729–1799), who studied male gametes, reported the immobilization of sperm placed on ice (Ledermann, 2020). This gave rise to the idea of freezing the sperm of Italian soldiers so that their wives, in the event of their husbands' death in war, could still have a child together.

It should be noted that until the first half of the 20th century, the effects of low temperatures on cells were mainly studied empirically. In 1938, B. Luyet (1892–1965) and E. Hodapp (1907–1996) reported the first successful attempt at vitrification of frog sperm using a 0.2 M sucrose solution (Luyet & Hodapp, 1938). B. Luyet was the first president of the World Society for Cryobiology. In 1940, he, together with his sister, published a book titled "Life and death at low temperatures".

By 1945, many scientists attempted to cryopreserve the sperm of various animal species, but they were unable to preserve its fertilizing ability. Significant cellular damage was caused by ice crystals formed during the cooling of cells. To solve this problem, specific substances were added to the solution in which the cells were frozen. These substances helped prevent the formation of ice crystals inside the cells and lowered the freezing temperature. Such substances are called cryoprotectants, and besides protecting against damage caused by cryopreservation factors, they must have low toxicity for the cells. Glycerol turned out to be an effective cryoprotectant for sperm, with its cryoprotective properties discovered in 1949. While studying the

movement of rooster sperm by adding a few drops of glycerol to a Petri dish, K. Polge (1926–2006) observed an unexpected effect from freezing sperm in liquid nitrogen – the cells remained viable. He described the result of his "accidental" observation in the scientific journal "Nature" (Polge, Smith, & Parkes, 1949). Like many other great discoveries, the protective property of glycerol was discovered by mistake. A sperm sample was treated with Meyer's solution, traditionally used to fix smears before staining, which was apparently mislabeled as fructose. Using glycerol, K. Polge and his colleagues successfully froze and thawed bird and bull sperm, achieving viable sperm cells after thawing. This breakthrough enabled the successful freezing and thawing of sperm without significant loss of viability, laying the foundation for the development of human sperm cryopreservation.

K. Polge's experiments were further developed by Dr. J. Sherman, who is considered the first to successfully cryopreserve human sperm. In 1953, he established the world's first cryobank. He was able to inseminate a woman with thawed donor sperm and achieve a successful pregnancy. At that time, the American legal system prohibited the insemination of women with donated sperm, as it was considered "contrary to public policy and good morals". Therefore, the news of the birth of a child using cryopreserved sperm was not disclosed until 10 years later (Sherman, 1963).

During this period, scientists were also studying the properties of other substances in reducing the destructive power of ice crystals during the freezing of biological specimens. In 1959, J. Lovelock (1919–2022) and M. Bishop (1922–1984) discovered the protective properties of dimethyl sulfoxide (DMSO) (Lovelock, 1954). The advantages of this new cryoprotectant lay in its greater permeability through cell membranes compared to glycerol. In 1964, a dedicated group of scientists founded the International Society for Cryobiology, aimed at promoting research on the effects of low temperatures on all organisms, organs, tissues, and cells (Pegg, 2002). One of the members of this society was the founder of reproductive cryobiology, P. Mazur (1928–2015). In the 1960s and 1970s, P. Mazur determined the permeability characteristics of cell membranes for water and cryoprotectant solutions (Pegg & Kleinhans, 2016). In 1972, P. Mazur outlined the main postulates and basic characteristics of the biological and physical processes that occur during the gradual freezing of living objects. This theoretical framework, which became known as the "two-factor theory of P. Mazur", states that there are two main factors that damage cells during freezing: 1) intracellular ice crystals; 2) high concentrations of salts, which increase when extracellular water begins to crystallize and the salt ions end up in the smaller volume of supercooled water outside the ice. Considering these factors, P. Mazur concluded that excessively rapid cooling destroys cells due to the formation of intracellular ice. On the other hand, excessively slow cooling damages cells due to the toxicity of increasing salt concentrations and mechanical external damage. Thus, P. Mazur's research results became the biophysical basis for experiments in the cryopreservation of human gametes and embryos. Based on the "two-factor theory", scientists have proven that the extent of cell damage depends on the permeability coefficient of the cell membrane for water molecules and cryoprotectants, as well as the cooling rate.

It was established that protocols optimal for one type of cell were ineffective for another. The methods for cryopreservation of mammalian reproductive cells could not be extrapolated to human gametes due to their distinct anatomical, physiological, and biochemical properties (Arav, 2014).

During the 1960s and 1970s, researchers refined cryopreservation methods for different cell types, optimizing freezing and thawing protocols. Sperm banks were created to provide long-term storage for individuals seeking to preserve their fertility. Over the decades, researchers improved cooling and warming methods, developed new cryoprotectants, and designed new cryocontainers, which expanded the applications of cryotechnologies. However, the problem of low fertilization rates after using cryopreserved sperm remained. Cryopreserved sperm was characterized by lower motility and fertilizing ability (see Figure 1).

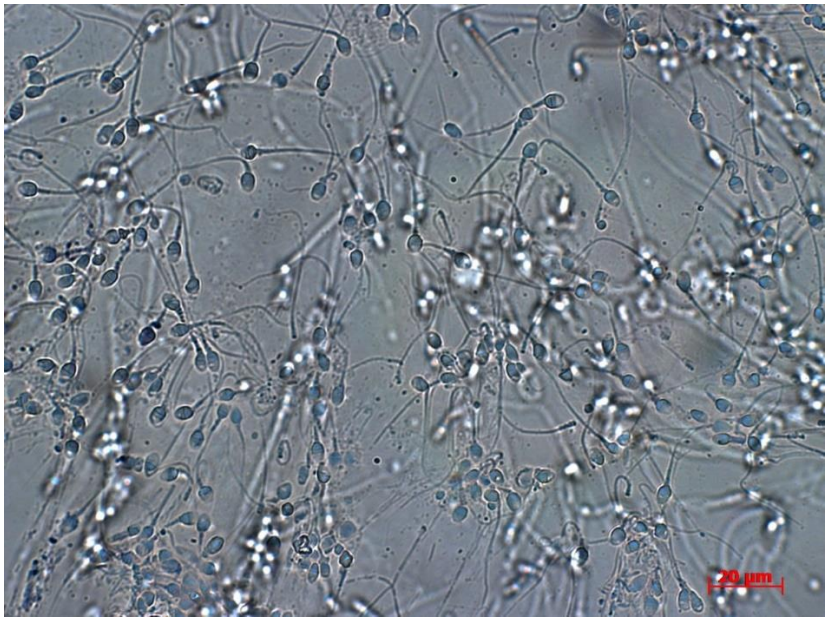

Figure 1. Human spermatozoa after cryopreservation (Author's source).

This issue was addressed in 1995 with the introduction of the innovative method of intracytoplasmic sperm injection (ICSI) (Palermo, Joris, Devroey, & Van Steirteghem, 1992).

This development not only enhanced the effectiveness of infertility treatments but also provided new ways to utilize genetic material previously considered infertile. The discovery of ICSI spurred the development of methods for cryopreservation of single sperm obtained through testicular and epididymal biopsy (see Figure 2). Such sperm were predominantly cryopreserved using the vitrification method. Vitrification is a rapid freezing technique that converts biological specimens into a glass-like state without forming ice crystals.

The first successful vitrification of human sperm was reported by a group of scientists from the University of Cologne (Nawroth et al., 2002). This vitrification technique led to the first pregnancies through intrauterine insemination of a patient with severe oligoasthenozoospermia, with the first live births worldwide recorded in 2012 (Isachenko, Rahimi, Mallmann, Sanchez, & Isachenko, 2011). Researchers

continue to investigate and propose new, original approaches to cryopreserving sperm from men with spermatogenesis pathology (Petrushko et al., 2021).

Figure 2. A Petri dish for ICSI (Author's source).

Cryopreservation of Human Oocytes and Pre-Implantation Embryos.

The technology for oocyte cryopreservation has developed over the past few decades, overcoming numerous scientific and technical challenges. The concept of oocyte cryopreservation emerged in the 1970s and 1980s. Early experiments focused on understanding the viability of oocytes after freezing and thawing (see Figure 3).

However, these initial attempts faced significant problems due to the unique cellular structure of oocytes, making them particularly sensitive to ice crystal formation and other damage during the freezing and thawing processes.

The first method developed for oocyte cryopreservation was based on slow freezing (see Figure 4).

This technique involved gradually lowering the temperature of oocytes in the presence of cryoprotectants. Despite some success, the slow freezing method often resulted in low survival rates due to ice crystal formation and cellular damage (Tharasanit & Thuwanut, 2021).

It wasn't until 1986 that a birth was reported following oocyte cryopreservation using the slow freezing method (Chen, 1986). The protocol involved gradually adding chilled DMSO to the oocytes, lowering the temperature to –7°C to induce ice formation, then further lowering the temperature to – 36°C at a rate of 0.5°C per minute. The oocytes were then transferred to liquid nitrogen at –196°C for storage. Despite significant progress with the slow freezing procedure, by the late 1990s, the pregnancy rate following embryo transfer from frozen oocytes was only 1–2% (Stachecki & Cohen, 2004). Due to the very low success rate of this method (5 pregnancies in 10 years), experimental research activity in this direction decreased.

Figure 3. Human oocyte after cryopreservation (Author's source).

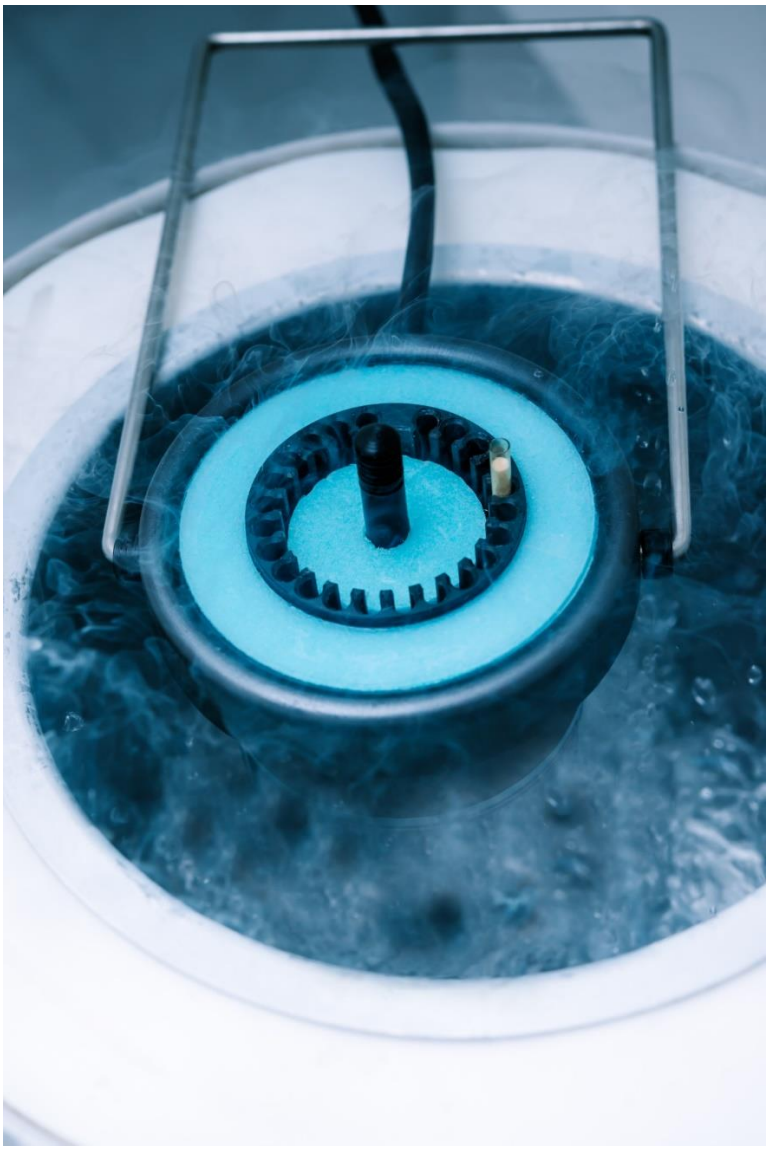

Figure 4. A cryochamber with a straw plunged in liquid nitrogen to oocytes slowly cool (Author's source).

The vitrification technique, which emerged later, represents a breakthrough in oocyte cryopreservation. This method has significantly improved the survival rates and developmental potential of cryopreserved oocytes, leading to higher pregnancy rates and better clinical outcomes. Researchers continue to explore and refine vitrification protocols to enhance their efficacy and reliability, making it a preferred method in reproductive medicine today (see Figure 5).

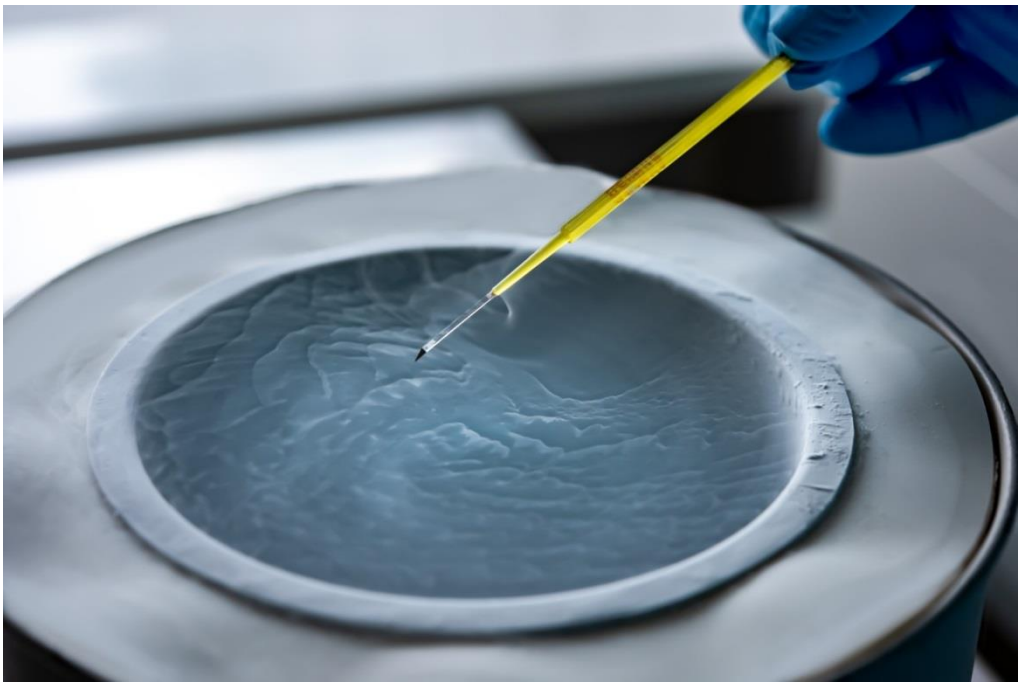

Figure 5. Cryodevice with oocytes before plunging into liquid nitrogen (Author's source).

The birth of a healthy girl was reported in 1999 (Kuleshova, Gianaroli, Magli, Ferraretti, & Trounson, 1999). Oocytes were vitrified in a highly concentrated cryoprotectant solution (40% w/v ethylene glycol and 0.6 mol/L sucrose) and placed into open straws, then submerged into the liquid nitrogen -196 °C. Rewarming was conducted in a sucrose solution at 37 °C.

The Cryotop method was developed in 2005, using an open carrier for the cryopreservation of both oocytes and embryos (Kuwayama, Vajta, Kato, & Leibo, 2005) (see Figure 6).

The Cryotop method became the preferred choice of many embryologists worldwide, with open carriers being the most widely used devices. By the early 2010s, vitrification according to the Kuwayama-methods had become the gold standard for oocyte cryopreservation.

Numerous fertility clinics worldwide began offering egg freezing services, and this technology was increasingly used for various clinical indications, including oncofertility (preservation of fertility for cancer patients) and elective fertility preservation (for women delaying childbirth for personal or professional reasons).

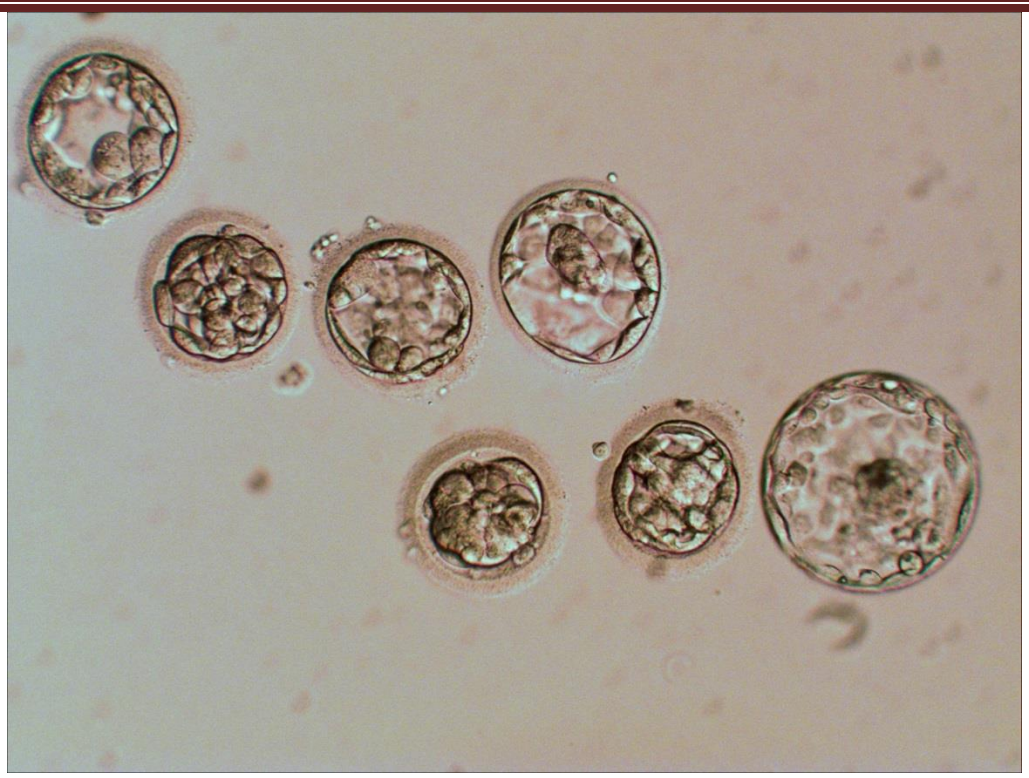

Figure 6. Human embryos of pre-implantation stages of development (Author's source).

Most cycles of infertility treatment using oocyte cryopreservation technology were conducted in Italy, as this country had laws prohibiting the freezing of human embryos (Porcu et al., 2004). In July 2006, a group of Italian scientists led by K. Oktay published a statistical analysis of the effectiveness of ART cycles with cryopreserved oocytes obtained from clinics in the country (Oktay, Cil, & Bang, 2006). They reported that the pregnancy rate following embryo transfer into the uterine cavity using slow frozenoocytes was 29.4%, while it was 51% with vitrified oocytes. A meta-analysis in 2006 of oocyte cryopreservation outcomes revealed a low incidence of pregnancies following ART cycles with frozen-thawed oocytes. The effectiveness of oocyte cryopreservation methods increased only in 2011, after five years of active fundamental research into cryodamage and cryoprotection of oocytes, the development of freezing and thawing protocols, and the search for safe cryoprotectants (Cobo & Diaz, 2011).

In 2000, the Department of Health in the United Kingdom allowed the use of frozen oocytes for infertility treatment but set a maximum storage period of 10 years in liquid nitrogen to prevent the negative impact of background radiation on the oocyte genome. In 2013, following the publication of results from randomized controlled trials conducted between 2008 and 2010 (Cobo, Meseguer, Remohí, & Pellicer, 2010; Rienzi et al., 2010), the American Society for Reproductive Medicine (ASRM) changed the status of oocyte cryopreservation technology from “experimental” to “clinical practice” due to identical main embryological parameters and pregnancy rates following embryo transfer into the uterine cavity using freshly retrieved and frozen-

thawed oocytes. This approval further confirmed the effectiveness of oocyte cryopreservation technology and encouraged its broader use.

Researchers have sought to improve existing cryopreservation protocols by attempting to automate the process (Brunetti et al., 2021). However, semi-automatic devices for gamete and embryo vitrification have not yet gained widespread adoption in clinical practice.

The history of embryo cryopreservation technology has been complex due to moral, ethical, legal, and religious controversies. The first pregnancy following the transfer of a slow frozen embryo was achieved as early as 1983 (Trounson & Mohr, 1983), although this pregnancy ended in miscarriage at ten weeks. The first child birth resulted from the transfer of a slow frozen embryo was on March 28, 1984, at the Queen Victoria Medical Centre in the United Kingdom (Zeilmaker et al., 1984). Ultra-rapid freezing of embryos was first applied in 1987 (Trounson, Peura, & Kirby, 1987). In 1985, the first child was born from a blastocyst embryo (Cohen et al., 1985), and in 1996, an embryo from a woman's natural cycle before chemotherapy was successfully cryopreserved (Brown, Modell, Obasaju, & King, 1996).

The history of reproductive cryomedicine has also seen unusual cases. For instance, in 2009, a 33-year-old American woman, Nadya Suleman, gave birth to six boys and two girls. Her doctor irresponsibly transferred twelve thawed embryos obtained from previous stimulated IVF cycles into her uterus. The California medical board deemed the transfer of such a large number of embryos a "practice that threatens life" and denied the doctor a license.

The history of reproductive cryobiology in Ukraine.

The birthplace of cryotechnology in Ukraine is undoubtedly the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (IPC&C NAS of Ukraine) on February 25, 1972. This institution is unique not only in Ukraine but worldwide, as it comprehensively develops and improves methods for hypothermic and low-temperature preservation of various types of cells, tissues, and even organs. Based on fundamental research results under the leadership of academician of the NAS of Ukraine Valentin Grischenko (1928–2011), the first service for artificial insemination with cryopreserved reproductive cells was established in Ukraine at the Department of Obstetrics and Gynecology of Kharkiv Medical University (Lytvynova, Trishch, & Terenda, 2023).

The first report of successful cryopreservation of a human embryo and the birth of a child was made in 2002 by a team of scientists from the cryobiology of reproduction systems department at the IPC&C NAS of Ukraine. In a favorable cycle, thawed embryos were transferred into the uterine cavity, and the patient became pregnant. Nine months later (on August 25, 2003), a healthy full-term boy was born (Grischenko, Petrushko, Gerodes, Terpyachaya, & Gurina, 2003).

Today, researchers at the IPC&C NAS of Ukraine are developing various methods to improve the outcomes of cryopreservation of sperm, oocytes, and human embryos, taking into account individual characteristics and the specific stages of their development (Petrushko, Yurchuk, Buderatska, & Piniaiev, 2018; Petrushko, Yurchuk,

Piniaiev, & Buderatska, 2019; Yurchuk, Likszo, Witek, Petrushko,& Skarzynski, 2024) (see Figure 7).

Figure 7. An employee of the IPC&C NAS of Ukraine working in cryo-storage (Author's source).

Every year, more than 30,000 children are born worldwide from cryopreserved oocytes and embryos (Han & Seifer, 2023).

The 21st century marked the first successful outcomes of cryopreservation of reproductive tissues. In Belgium, after autologous orthotopic transplantation of cryopreserved ovarian cortex, a healthy child was born (Donnez et al., 2011). However, the scientific community questioned the source of pregnancy. It was unclear whether the pregnancy resulted from fertilization of oocytes from the transplanted tissue or from

the reactivation of the dormant ovary. In 2000, scientists first transplanted human ovarian tissue after freezing and prolonged storage (Oktay & Karlikaya, 2000). In 2004 reported the first childbirth after orthotopic transplantation of cryopreserved ovarian tissue (Donnez et al., 2004). In 2006, a group of American scientists successfully cryopreserved human ovaries with their vascular pedicle (Bedaiwy, Hussein, Biscotti, & Falcone, 2006).

The successful cryopreservation of human testicular tissues was first reported by Belgian scientists. They demonstrated that thawed testicular tissue could maintain cellular ultrastructure, tubule morphology, and tissue function (Baert et al., 2013).

The prospects for the use of cryotechnologies in the field of ART are primarily seen in genetic diagnostics and genome editing. Cryotechnologies can be employed for the long-term preservation of DNA samples, cells, or embryos, allowing for genetic testing and diagnostics at any stage. This enables the detection of genetic diseases or mutations even before birth, which is crucial for treatment planning (Simpson, Kuliev, & Rechitsky, 2019). Additionally, cryotechnologies can be integrated into genome editing processes, such as CRISPR/Cas9, to preserve edited cells or embryos, positively impacting reproductive health and the development of personalized medicine.

Thus, the history of the development of cryotechnologies in reproductive medicine demonstrates significant progress from experimental research to widespread clinical application. Despite the successes achieved, questions regarding the efficiency and safety of these methods remain the subject of active scientific debate.

One of the main issues is the impact of cryopreservation on the long-term viability of gametes and embryos. Despite significant progress since the discovery of the cryoprotective properties of glycerol and other substances, cryopreservation is not an entirely risk-free procedure. The reasons for this are related to various scientific aspects, including physical, biochemical, and genetic phenomena that occur during the freezing and thawing of cells.

Additionally, the scientific community continues to discuss the ethical considerations of using cryotechnologies in reproductive medicine. Notably, the issue of storing and disposing of surplus embryos not used in ART cycles is a topic of debate (Ethics Committee of the ASRM, 2021).

Another important aspect of the discussion is the social and legal implications of using cryotechnologies. For example, the cryopreservation of oocytes to preserve fertility in women who delay childbirth for personal or professional reasons elicits mixed reactions. On the one hand, this method provides women with greater freedom in planning their lives, but on the other, it raises questions about social expectations and pressures that may compel women to freeze oocytes due to fears of age-related fertility decline (Bolton, Hayden, Robinson, Abdo, & Pericleous-Smith, 2023).

Despite all achievements, cryotechnologies remain a field for ongoing scientific research, ethical debate, and legal regulation. Therefore, it is important to continue research in this area, with the goal of not only improving fertility preservation methods but also ensuring their safety and ethical justification.

Thus, in the context of the historical foundations of reproductive cryobiology, our study highlights certain aspects that should be considered when analyzing key stages in the development of this field. Specifically, we place greater emphasis on the influence of historical circumstances and scientific discoveries on the formation of cryopreservation approaches, whereas other authors (Iussig et al., 2019) focus on specific technical aspects. This distinction underscores the multifaceted nature of the topic under study and the need for a comprehensive approach to researching the history of cryobiology methods in reproductive medicine.

We hope that in the future, scientists will make many more discoveries in the field of cryopreservation of reproductive cells, pre-implantation embryos, tissues, and organs. Fundamental research in cryobiology and cryomedicine, molecular biology, genetics, physics, biochemistry, and physiology will uncover new mechanisms of cryoresistance and cryosensitivity of bioobjects at different levels of organization and create new cryotechnologies to ensure the birth of a healthy child.

Conclusions.

Based on the study, it can be concluded that cryotechnology has played a decisive role in the advancement of reproductive medicine. The history of cryopreservation of sperm, oocytes, and preimplantation human embryos illustrates the relentless pursuit of scientific innovation, evolving from the randomness of early accidental successes to the stability of today's sophisticated vitrification techniques. Each new and pioneering approach to cryopreservation has brought significant improvements in fertility preservation and the effectiveness of ART. This evolution has enabled millions of people to fulfill their dream of having healthy children. As cryotechnology continues to progress, future advancements promise not only to further enhance the effectiveness and accessibility of cryopreservation but also to offer renewed hope for those aspiring to parenthood.

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Conflicts of interest.

The authors declare no conflict of interest.

References

Arav, A. (2014). Cryopreservation of oocytes and embryos. *Theriogenology*, *81*(1), 96–102. https://doi.org/10.1016/j.theriogenology.2013.09.011

Baert, Y., Van Saen, D., Haentjens, P., In't Veld, P., Tournaye, H., & Goossens, E. (2013). What is the best cryopreservation protocol for human testicular tissue banking? *Human Reproduction*, *28*(7), 1816–1826. https://doi.org/10.1093/humrep/det100

Bedaiwy, M. A., Hussein, M. R., Biscotti, C., & Falcone T. (2006). Cryopreservation of intact human ovary with its vascular pedicle. *Human Reproduction, 21*(12), 3258–3269. https://doi.org/10.1093/humrep/del227

Bolton, V. N., Hayden, C., Robinson, M., Abdo, D., & Pericleous-Smith, A. (2023). Association of reproductive and clinical scientists and British fertility society. Human oocyte cryopreservation: revised evidence for practice. *Human Fertility(Camb), 26*(1), 2–16. https://doi.org/10.1080/14647273.2023.2190987

Brown, J. R., Modell, E., Obasaju, M., & King, Y. K. (1996). Natural cycle in-vitro fertilization with embryo cryopreservation prior to chemotherapy for carcinoma of the breast. *Human Reproduction, 11*(1), 197–199. https://doi.org/10.1093/oxfordjournals.humrep.a019017

Brunetti, X. O., Cawood, S., Gaunt, M., Saab, W., Serhal, P., & Seshadri, S. (2021). The first livebirth using warmed oocytes by a semi-automated vitrification procedure. *Journal of Reproduction & Infertility, 22*(1), 70–72. https://doi.org/10.18502/jri.v22i1.4998

Chen, C. (1986). Pregnancy after human oocyte cryopreservation. *Lancet, 1*(8486), 884–886. https://doi.org/10.1016/s0140-6736(86)90989-x

Cobo, A., & Diaz, C. (2011). Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. *Fertility and Sterility*, *96*(2), 277–285. https://doi.org/10.1016/j.fertnstert.2011.06.030

Cobo, A., Meseguer, M., Remohí, J., & Pellicer, A. (2010). Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Human Reproduction*, *25*(9), 2239–2246. https://doi.org/10.1093/humrep/deq146

Cohen, J., Simons, R. F., Fehilly, C. B., Fishel, S. B., Edwards, R. G., Hewitt, J., … Webster, J. M. (1985). Birth after replacement of hatching blastocyst cryopreserved at expanded blastocyst stage. *Lancet*, *1*(8429), 647. https://doi.org/10.1016/s0140-6736(85)92194-4

Donnez, J., Dolmans, M. M., Demylle D., Jadoul, P., Pirard, C., Squifflet, J., ... van Langendonckt, A. (2004). Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet, 364*(9443), 1405–1410. https://doi.org/10.1016/S0140-6736(04)17222-X

Donnez, J., Silber, S., Andersen, C. Y., Demeestere, I., Piver, P., Meirow, D., … Dolmans, M. M. (2011). Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. *Annals of Medicine, 43*(6), 437–450. https://doi.org/10.3109/07853890.2010.546807

Ethics Committee of the American Society for Reproductive Medicine. Disposition of unclaimed embryos: an Ethics Committee opinion. (2021). *Fertility and Sterility, 116*(1), 48–53. https://doi.org/10.1016/j.fertnstert.2021.02.020

Giuliano, R., Maione, A., Vallefuoco, A., Sorrentino, U., & Zuccarello, D. (2023). Preimplantation genetic testing for genetic diseases: limits and review of current literature. *Genes (Basel)*, *14*(11), 2095. https://doi.org/10.3390/genes14112095

Grischenko, V., Petrushko, M., Gerodes, A., Terpyachaya, I., & Gurina, T. (2003). Pregnancy and labour after transplantation of frozen-thawed human embryos. *Problems of Cryobiology and Cryomedicine*, *13*(3), 99–102. Retrieved from http://www.journal.cryo.org.ua/index.php/probl-cryobiol-cryomed/article/download/1066/1150

Han, E., & Seifer, D. B. (2023). Oocyte cryopreservation for medical and planned indications: A practical guide and overview. *Journal of Clinical Medicine*, *12*(10), 3542. https://doi.org/10.3390/jcm12103542

Isachenko, E., Rahimi, G., Mallmann, P., Sanchez, R., & Isachenko, V. (2011). Novel approaches to the cryopreservation of human spermatozoa: History and development of the spermatozoa vitrification technology. *Journal Reproductive Stem Cell Biotechnology*, *2*(2), 128–145. https://doi.org/10.1177/205891581100200207

Iussig, B., Maggiulli, R., Fabozzi, G., Bertelle, S., Vaiarelli, A., Cimadomo, D., ... Rienzi, L. (2019). A brief history of oocyte cryopreservation: arguments and facts. *Acta Obstetricia et Gynecologica Scandinavica*, *98*(5), 550–558. https://doi.org/10.1111/aogs.13569

Kuleshova, L., Gianaroli, L., Magli, C., Ferraretti, A., & Trounson, A. (1999). Birth following vitrification of a small number of human oocytes: Case Report. *Human Reproduction*, *14* (12), 3077–3079. https://doi.org/10.1093/humrep/14.12.3077

Kuwayama, M., Vajta, G., Kato, O., & Leibo, S. P. (2005). Highly efficient vitrification method for cryopreservation of human oocytes. *Reproductive BioMedicine Online*, *11*(3), 300–308. https://doi.org/10.1016/S1472-6483(10)60837-1

Ledermann, D. W. (2020). Reading Spallanzani today. *Revista Chilena de Infectología*, *37*(1), 64–68. https://doi.org/10.4067/S0716-10182020000100064

Lovelock, J. E. (1954). Biophysical aspects of the freezing and thawing of living cells. *Proceedings of the Royal Society of Medicine*, *47*(1), 60–62. PMID:13134172

Luyet, B. J., & Hodapp, E. L. (1938). Revival of frog's spermatozoa vitrified in liquid air. *Proceedings of the Society for Experimental Biology and Medicine*, *39*(3), 433–434. https://doi.org/10.3181/00379727-39-10229P

Lytvynova, N. O., Trishch, N. M., & Terenda, N. O. (2023). Istorychni aspekty akusherstva yak osnova formuvannia ta rozvytku akusherskykh naukovykh shkil [Historical aspects of obstetrics as the basis for the formation and development of obstetric scientific schools]. *Visnyk sotsialnoi hihiieny ta orhanizatsii okhorony zdorovia v Ukraini [Bulletin of social hygiene and health care organization in Ukraine]*, *3*(97), 98–104. https://doi.org/10.11603/1681-2786.2023.3.14230 [in Ukrainian]

Nawroth, F., Isachenko, V., Dessole, S., Rahimi, G., Farina, M., Vargiu, N., … Isachenko, E. (2002). Vitrification of human spermatozoa without cryoprotectants. *Cryo Letters*, *23*(2), 93–102. PMID: 12050777

Oktay, K., Cil, A. P., & Bang, H. (2006). Efficiency of oocyte cryopreservation: a meta-analysis. *Fertility and Sterility*, *86*(1), 70–80. https://doi.org/10.1016/j.fertnstert.2006.03.017

Oktay, K., & Karlikaya, G. (2000). Ovarian function after transplantation of frozen, banked autologous ovarian tissue. *The New England Journal of Medicine*, *342*(25), 1919. https://doi.org/10.1056/NEJM200006223422516

Palermo, G., Joris, H., Devroey, P., & Van Steirteghem, A. C. (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, *340*(8810),17–18. https://doi.org/10.1016/0140-6736(92)92425-f

Pegg, D. E. (2002). The history and principles of cryopreservation. *Seminars in Reproductive Medicine, 20*(1), 5–13. https://doi.org/10.1055/s-2002-23515

Pegg, D., & Kleinhans, F. (2016). In memoriam Peter Mazur – cryobiologist. *Cryobiology*, *72*(2), 83–5. https://doi.org/10.1016/j.cryobiol.2016.02.004.

Petrushko, M., Piniaiev, V., & Yurchuk, T. (2021). The history of assisted reproductive technologies: from prohibition to recognition. *History of Science and Technology*, *11*(2), 315–328. https://doi.org/10.32703/2415-7422-2021-11-2-315-328

Petrushko, M., Yurchuk, T., Piniaiev, V., & Buderatska N. (2019). Cryopreservation of incomplete compacted morulae and preliminary biopsy of excluded fragments. *Zygote*, *27*(6), 386–391. https://doi.org/10.1017/S0967199419000455

Petrushko, M., Yurchuk, T., Todorov, P., Hristova, E., Piniaiev, V., Isachenko, E., … Isachenko, V. (2021). New method for cryoprotectant-free freezing of human oligoasthenoteratozoospremic spermatozoa with high-molecular polymer. *Cryobiology, 103*, 39–44. https://doi.org/10.1016/j.cryobiol.2021.09.013

Petrushko, M., Yurchuk, T., Buderatska, N., & Piniaiev, V. (2018). Oolemma invagination of fresh and cryopreserved human oocytes during in vitro fertilization by ICSI. *Problems of Cryobiology and Cryomedicine*, *28*(3), 258–265. https://doi.org/10.15407/cryo28.03.258

Polge, C., Smith, A. U., & Parkes, A. S. (1949). Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature, 164*, 666. https://doi.org/10.1038/164666a0

Porcu, E., Fabbri, R., Damiano, G., Fratto, R., Giunchi, S., & Venturoli, S. (2004). Oocyte cryopreservation in oncological patients. *European Journal of Obstetrics and Gynecology and biology, 5*(113), 14–16. https://doi.org/10.1016/j.ejogrb.2003.11.004

Rienzi, L., Romano, S., Albricci, L., Maggiulli, R., Capalbo, A., Baroni, E., … Ubaldi, F. (2010). Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. *Human Reproduction, 25*(1), 66–73. https://doi.org/10.1093/humrep/dep346

Sherman, J. (1963). Questionable protection by intracellular glycerol during freezing and thawing. *Journal of Cellular Physiology, 61*, 67–83. https://doi.org/10.1002/jcp.1030610108

Simpson, J. L., Kuliev, A., & Rechitsky S. (2019). Overview of preimplantation genetic diagnosis (PGD): historical perspective and future direction. *Methods Molecular Biology, 1885*, 23–43. https://doi.org/10.1007/978-1-4939-8889-1_2

Smeets, H. J. M., Sallevelt, S. C. H. E. N., & Herbert, M. (2023). Reproductive options in mitochondrial disease. *Handbook of Clinical Neurology*, *194*, 207–228. https://doi.org/10.1016/B978-0-12-821751-1.00004-X

Stachecki, J. J., & Cohen, J. (2004). An overview of oocyte cryopreservation. *Reproductive BioMedicine Online. 9*(2), 152–163. https://doi.org/10.1016/s1472-6483(10)62124-4

Tharasanit, T., & Thuwanut, P. (2021). Oocyte cryopreservation in domestic animals and humans: principles, techniques and updated outcomes. *Animals (Basel)*, *11*(10), 2949. https://doi.org/10.3390/ani11102949

Trounson, A., & Mohr, L. (1983). Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature*, *305*(5936), 707–709. https://doi.org/10.1038/305707a0

Trounson, A., Peura, A., & Kirby, C. (1987). Ultrarapid freezing: a new low-cost and effective method of embryo cryopreservation. *Fertility and Sterility*, *48*(5), 843–850. https://doi.org/10.1016/s0015-0282(16)59542-9

Yurchuk, T., Likszo, P., Witek, K., Petrushko, M., & Skarzynski D. J. (2024). New approach to the cryopreservation of GV oocytes and cumulus cells through the lens of preserving the intercellular gap junctions based on the bovine model. *International Journal of Molecular Sciences*, *25*(11), 6074. https://doi.org/10.3390/ijms25116074

Zeilmaker, G. H., Alberda, A. T., van Gent, I., Rijkmans, C. M., & Drogendijk, A. C. (1984). Two pregnancies following transfer of intact frozen-thawed embryos. *Fertility and Sterility, 42*(2), 293–296. https://doi.org/10.1016/s0015-0282(16)48029-5

Zhu, Y. (2022). Advances in CRISPR/Cas9. *BioMed Research International*, 9978571. https://doi.org/10.1155/2022/9978571

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Історія кріотехнологій у репродуктивній медицині: Від випадковості до стабільності

Анотація. *Стаття містить огляд історичних віх кріобіології, наукової галузі, яка знайшла широке практичне застосування в репродуктивній медицині. Кріотехнології зробили революцію в допоміжних репродуктивних технологіях, надаючи безцінні інструменти для зберігання, захисту та управління репродуктивними клітинами, такими як сперматозоїди, ооцити та ембріони. Технологія почалася з перших успішних спроб кріоконсервування сперматозоїдів, які не тільки продемонстрували можливість зберігання чоловічих статевих клітин, але й надихнули хвилю досліджень, спрямованих на розробку протоколів*

кріоконсервації більш чутливих і складних біологічних об'єктів, включаючи ооцити та передімплантаційні ембріони. Кріоконсервування стало важливим компонентом збереження фертильності, пропонуючи нові можливості для окремих осіб і пар, які стикаються з медичним лікуванням, яке може поставити під загрозу їхній репродуктивний потенціал, а також надаючи варіанти для відстрочення батьківства. Враховуючи критичну роль кріотехнологій у сучасній репродуктивній медицині, ця стаття заглиблюється в історичний контекст цих подій, досліджуючи ключові прориви, які сформували цю сферу. Автори провели поглиблений аналіз існуючої літератури, спираючись на широкий спектр наукових баз даних, щоб підкреслити глобальний вплив кріобіології на сьогоденні успіхи репродуктивної медицини. Крім того, у статті представлено результати власних досліджень та практичний досвід авторів у галузі репродуктивної кріобіології з особливим акцентом на застосуванні цих технологій в Україні. Огляд підкреслює проблеми та можливості, які виникали протягом історії кріоконсервації, а також поточні зусилля щодо вдосконалення та оптимізації цих методів для подальшого покращення результатів лікування безпліддя. Обговорення також стосується етичних і матеріально-технічних міркувань, пов'язаних із кріоконсервуванням, зокрема в контексті тривалого зберігання та майбутнього використання кріоконсервованих матеріалів. Оскільки кріобіологія продовжує розвиватися, її інтеграція в репродуктивну медицину, безсумнівно, призведе до подальших інновацій, зробивши її наріжним каменем лікування безпліддя та репродуктивного здоров'я в усьому світі.

Ключові слова: *кріоконсервація; вітрифікація; повільне заморожування; розморожування; екстракорпоральне запліднення; допоміжні репродуктивні технології*

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