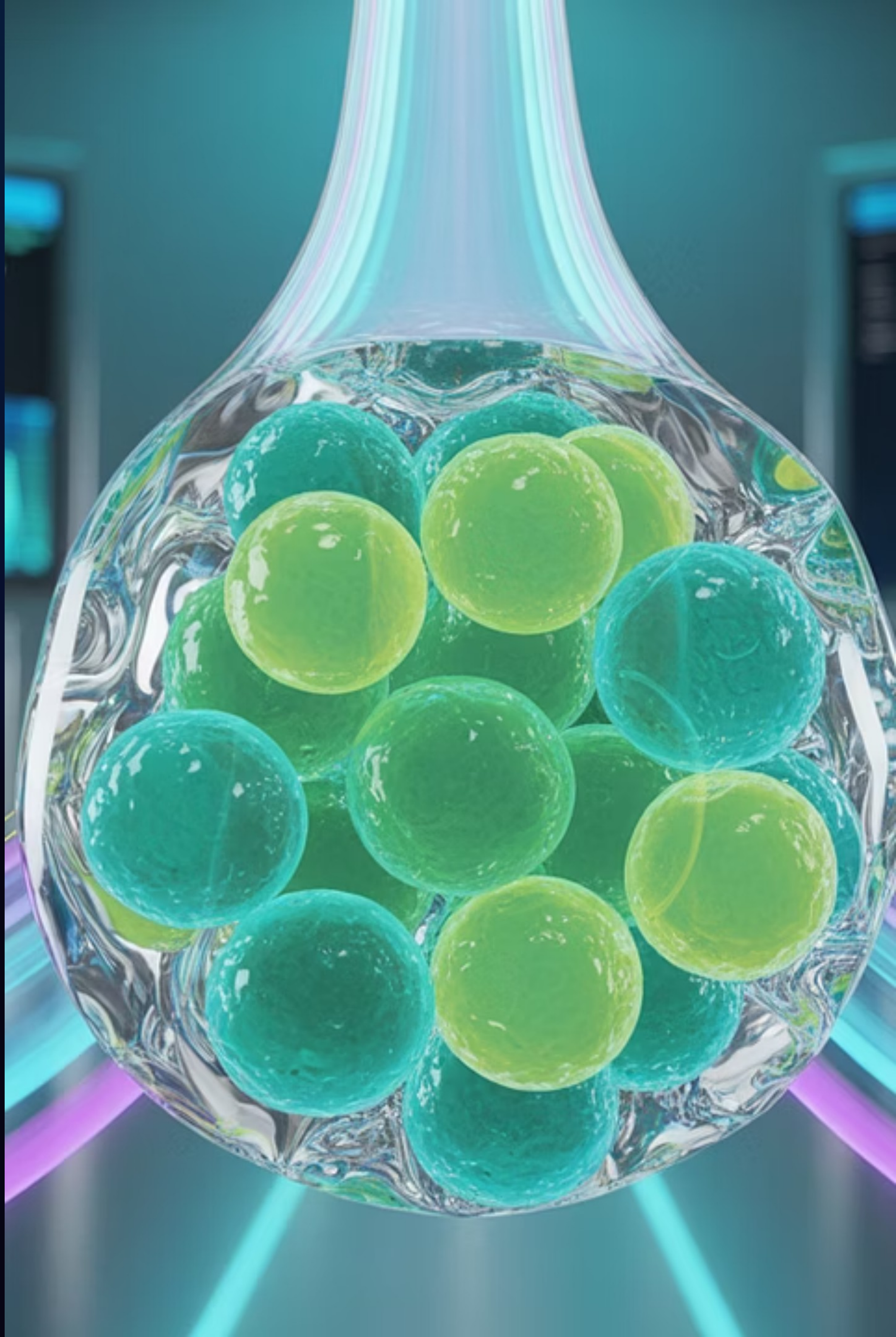
The Pioneering Past and Future of Vitrification

A comprehensive look at the history, development, and future applications of vitrification technology in reproductive medicine.



The Origins of Kinetic Vitrification

The concept of kinetic vitrification is credited to Father Basile J Luyet, a Professor of Biology at Saint Louis University. Luyet reviewed historic research in cryobiology and published summaries of experiments which mostly involved freezing under natural conditions, without cryoprotective agents (CPAs).

Luyet showed that supercooled solutions could become so viscous that they solidified without crystallization, forming a transparent glass state, which he termed "vitrification." He determined that although successful vitrification could occur during cooling, it did not guarantee cellular survival upon warming due to potential recrystallization.

In the 1950s, Gonzales and Luyet experienced limited success vitrifying chick hearts

Early Pioneers in Vitrification Research

1

Late 1970s - Early 1980s

Drs. Greg Fahy and Bill Rall independently began exploring vitrification of living tissue without ice formation. Fahy focused on whole tissue/organ preservation, while Rall studied ice formation inside cells during conventional freezing procedures.

2

1983

Fahy and Rall formalized their scientific union through the support of Harold Meryman, Scientific Director at the American Red Cross Blood Research Laboratory in Bethesda, MD.

3

1985

Under cold room experimental conditions, they successfully vitrified mouse embryos, marking a significant breakthrough in the field.

4

By 1990

A new focus on vitrification began, emphasizing minimizing the potential toxicity of vitrification solutions through combined use of mixed permeating CPAs and non-permeating solutes.

These early pioneers laid the groundwork for modern vitrification techniques that would eventually transform reproductive medicine.

Integration into Clinical IVF

Nearly a decade passed before vitrification was proposed as a serious technology for improving cryopreservation of human oocytes, cleaved embryos and blastocysts. This interest in clinical vitrification was linked to the formation of unique cryodevices such as:

- Open Pulled Straw (late 1990s)
- Cryoloops (early 2000s)
- Cryotops (early 2000s)

These devices featured thin surrounding films of vitrification solution and direct contact with liquid nitrogen (LN2) to achieve ultrarapid cooling rates.

By the mid-2000s, commercial industry development of new devices (HSV, Cryotip, Rapid-i

Open vs. Closed Vitrification Systems

Open Systems

Direct exposure to liquid nitrogen (LN2) achieves ultrarapid cooling rates. Initially believed necessary for high survival rates. Majority of oocyte cryopreservation experience supports their use, but potential contamination concerns exist.

Closed Systems

Slower cooling rates but provide aseptic conditions. Proven equally effective for embryo cryopreservation. Mixed results reported for oocyte vitrification, but continued development shows promise. Examples include HSV, Vitrisafe, microSecure, and SafeSpeed.

Key Finding

Research by Seki and Mazur proved that rapid to ultrarapid warming is the key determinant overriding conditions created at any cooling rate. The warming rate must be greater than the cooling rate for optimal success.

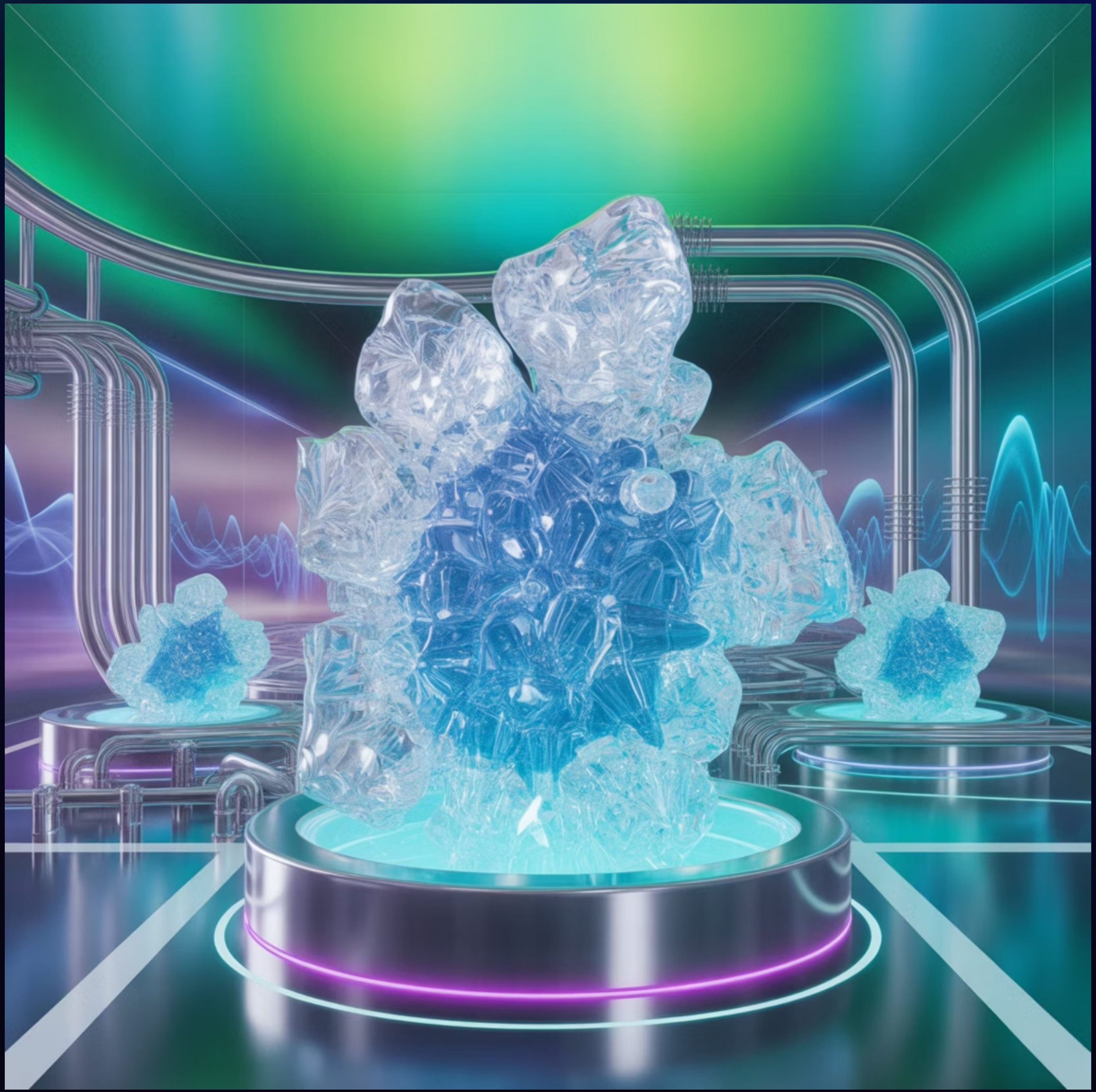
The debate between open and closed systems continues, with each having advantages depending on the specific application and tissue type being preserved.

The Importance of Warming Rates

During the development of vitrification technology, the relative importance of warming rates to ensuring successful vitrification was proven in a murine experimental model system by Seki and Mazur (the father of modern cryobiology).

Using a nonequilibrium, unstable vitrification solution model, they clearly proved that rapid to ultrarapid warming is the key determinant overriding conditions created at any cooling rate.

> "The faster one cools, the smaller the size of the invisible extracellular crystals in solution; the smaller the nucleated crystals are the greater their driving force to increase in size upon recrystallization during warming."



Vitrification warming is a complex process. A closed device that achieves intermediate

Transformation of the IVF Industry

Although it took more than 20 years of development, vitrification has transformed the IVF industry in several key areas:

Oocyte Cryobanking

Enabled effective preservation of female fertility, creating new options for women facing medical treatments or desiring delayed childbearing

Freeze-All IVF Cycles

Justified adoption of freeze-all approaches in conjunction with blastocyst culture and micromanipulation, improving outcomes.

Genetic Testing

Facilitated blastocyst biopsy/preimplantation genetic screening (PGS) with single embryo transfer applications, achieving over 99% survival rates.

Improved Success Rates

Enabled efficient pregnancy success across all age groups following single euploid embryo transfer.

With these advancements, vitrification has become the most significant procedure applied to assisted reproductive technology since the development of intracytoplasmic sperm injection (ICSI).

Blastocyst Vitrification Success

The development and clinical application of blastocyst vitrification has experienced the greatest success in terms of maintaining the viability of fresh embryos, proving superior to conventional slow freezing.

Post-warming, blastocysts tend to appear completely intact, with the occasional appearance of a few necrotic outer trophectodermal cells. Complete blastocyst survival rates routinely exceed 95%, and vitrified embryo transfer (VFET) live birth outcomes are routinely equal to or higher than fresh embryo transfer success.

To promote high blastocyst survival, pre-vitrification blastocoel collapsing (fluid volume

Blastocyst Vitrification Techniques

Blastocoel Collapsing

Artificial collapsing of blastocysts prior to vitrification has been proven effective using various methods. This reduces fluid volume to enhance post-warming viability.

Genetic Testing

Single euploid vitrified/warmed blastocyst transfer, independent of age, is the most efficient way to achieve high implantation and live birth rates.

Laser Ablation

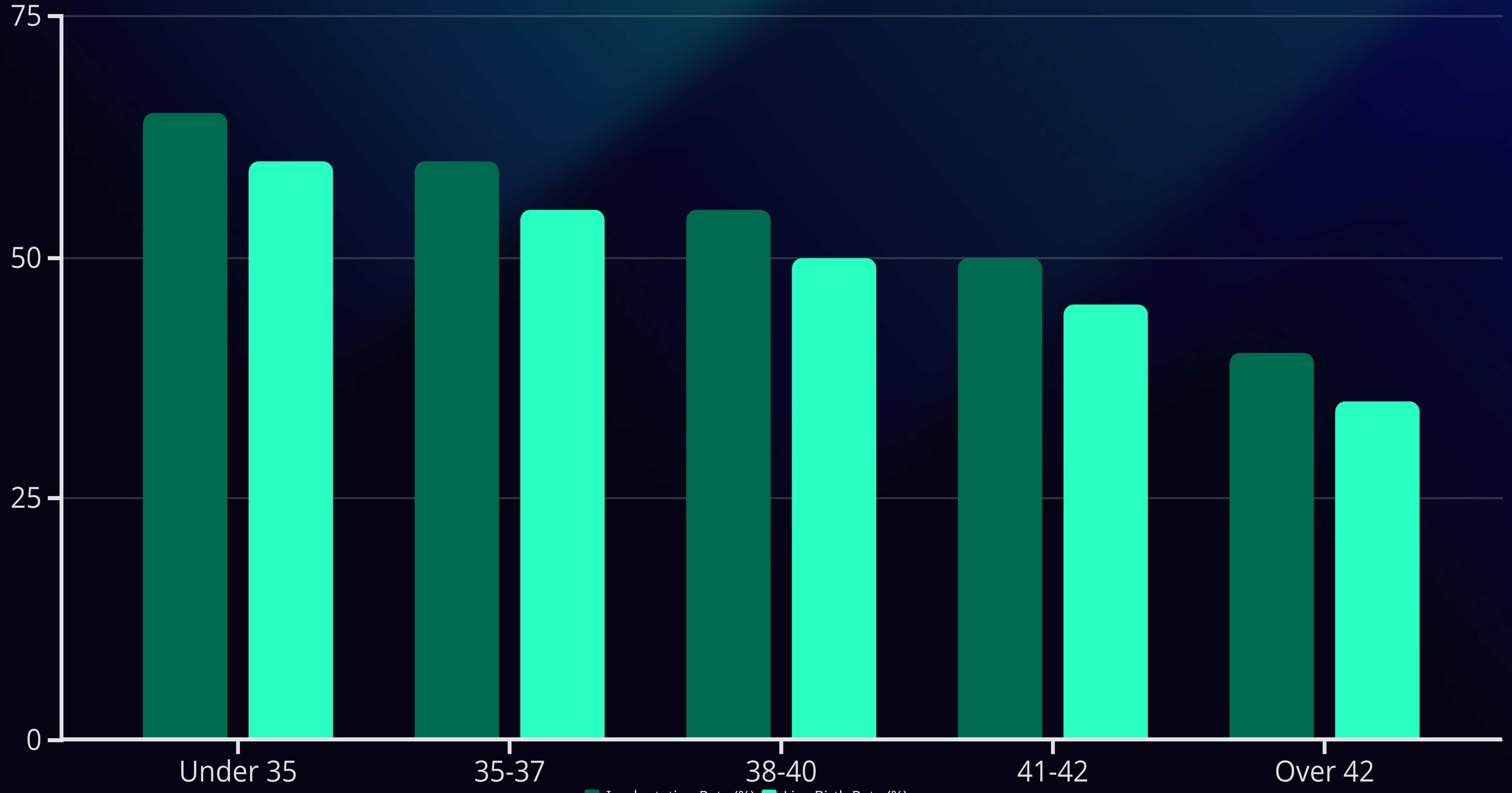
Trophectoderm laser ablation is the simplest approach, sacrificing a single cell for the greater good of the whole embryo.

CPA Selection

The need to manually collapse a blastocyst depends on the type and concentration of cryoprotective agents (CPAs) used. Glycerol-based solutions may not require artificial collapsing.

These techniques have contributed to making blastocyst vitrification a highly reliable procedure with exceptional clinical outcomes.

Clinical Outcomes of Vitrified Embryo Transfer



Oocyte Vitrification Development

1. Early 2000s

Breakthrough efforts in early oocyte slow freezing success integrated ICSI to reliably achieve fertilization and blastocyst development, leading to healthy live births.

2. Mid-2000s

Early success in vitrifying and warming human oocytes transformed reproductive practice into a highly efficient freeze preservation method.

3. 2013

After verifying the normal health of over 900 babies produced from embryos derived from slow-frozen and vitrified oocytes, the American Society for Reproductive Medicine (ASRM) reclassified oocyte cryopreservation technology as "nonexperimental."

4. 2013-Present

Rapid growth in commercial egg banking and fertility preservation services, with continued refinement of techniques to improve outcomes.

The development of oocyte vitrification has provided crucial options for fertility preservation, especially for women facing potential sterilizing medical treatments.

Applications of Oocyte Vitrification

Medical Fertility Preservation

The most important application is providing a "fertility preservation" option for women undergoing potential sterilizing medical treatments or facing medical uncertainty during their fertile years.

Emergency IVF Rescue

In emergency IVF cycle situations where sperm is unavailable on the day of oocyte retrieval (failed testicular biopsy or ejaculatory failure), vitrification provides an effective solution to rescue the cycle.

Commercial Egg Banking

The commercial banking and marketing of donor oocytes is the fastest growing and largest application of oocyte cryopreservation technology, creating new options for patients requiring donor eggs.

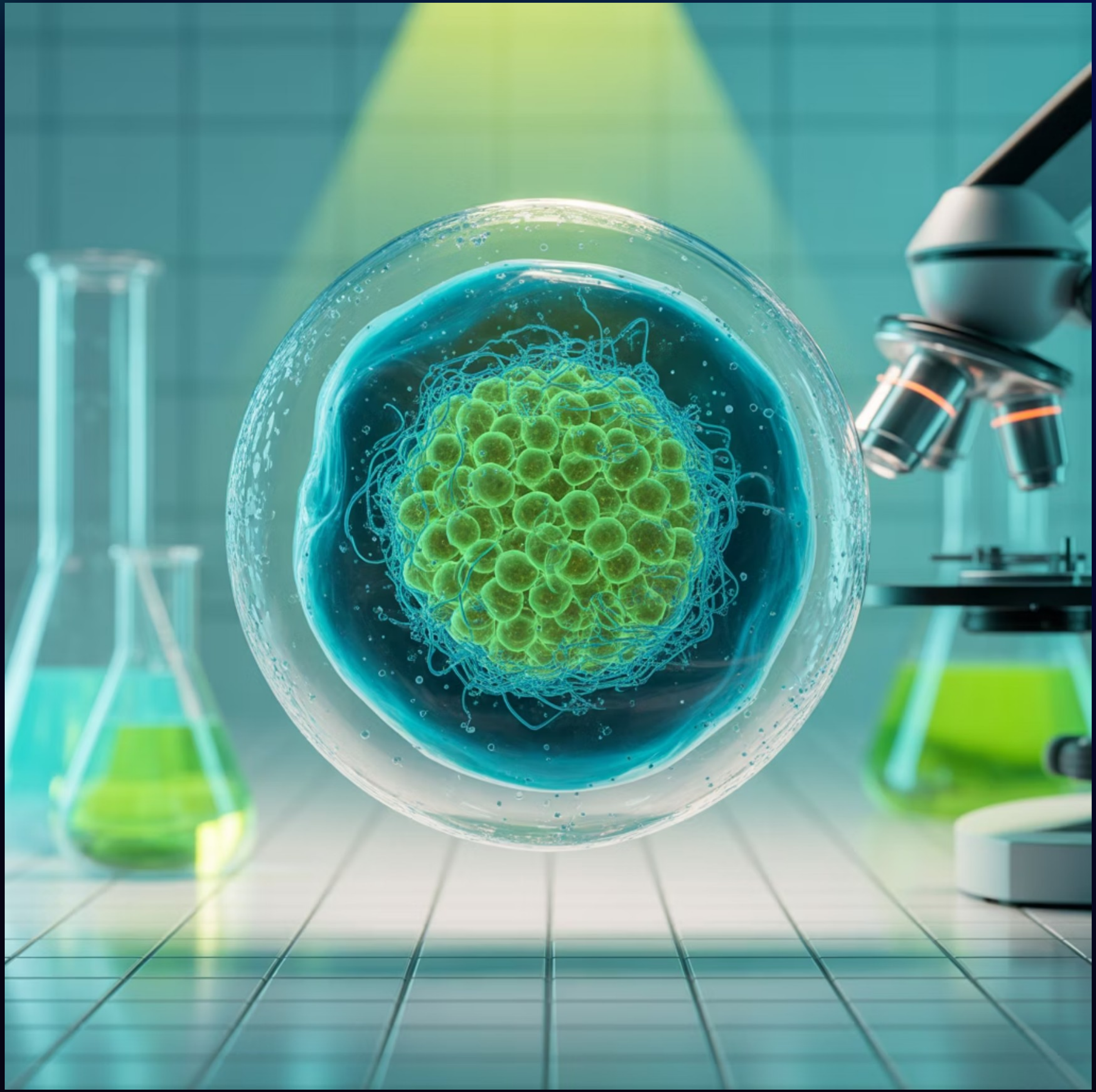
Elective Fertility Preservation

Women choosing to delay childbearing for personal or career reasons can preserve their eggs at a younger age when they are more viable, though success rates vary by age at freezing.

Challenges in Oocyte Vitrification

Despite significant progress, oocyte vitrification faces several challenges related to the special structure and sensitivity of this large single cell:

- Low permeability coefficient of the oolemma makes penetration of cryoprotectants difficult
- Intracytoplasmic lipids make oocytes more sensitive to freezing than embryos
- Potential precocious oocyte activation induced by exposure to cryoprotectants
- Loss of high mitochondrial polarity affecting calcium regulation
- Microvacuolization in the ooplasm and ultrastructural alterations



Research has shown that vitrification causes less damage to meiotic spindle integrity and chromosome alignment compared to slow freezing, with spindle recovery occurring more rapidly (1-2 hours post

Oocyte Vitrification Outcomes

88%

Survival Rate

Percentage of vitrified oocytes that survive the warming process intact.

78%

Fertilization Rate

Percentage of warmed oocytes that successfully fertilize following ICSI.

52%

Clinical Pregnancy

Clinical pregnancy rate using vitrified donor eggs reported by My Egg Bank.

<2%

Genetic Anomalies

Rate of genetic anomalies in babies derived from vitrified oocytes, similar to fresh oocytes.

While these statistics show promising results, there remains a gap between vitrified and fresh oocyte success rates. Fresh donor egg cycles in the USA typically attain an average 55% live birth rate, with top programs achieving 65-85% live birth rates. In contrast, commercial egg banks repeatedly report sub-50% live birth rates.

Human Ovarian Tissue Vitrification

Gosden et al were the first to explore ovarian tissue cryopreservation using slow freezing methods with sheep ovaries. Similar freeze preservation success has subsequently been achieved in humans using both slow freezing and vitrification methods.

Comparative vitrification solution trials have been initiated to identify optimal solutions for ovarian tissue freeze preservation. Promising vitrification results have also been attained in a Macaque monkey model using a metastable solution composed of 25% EG, 25% glycerol and polymers in a closed system device.

There have been promising developments in the cryopreservation of whole organs by

Ovarian Tissue Preservation Applications

Fertility Preservation for Cancer Patients

Ovarian tissue cryopreservation is a viable option for patients who require immediate gonadotoxic medical treatment and cannot delay for oocyte or embryo cryopreservation.

Prepubertal Fertility Preservation

It is the only option available for prepubertal girls facing treatments that may damage their future fertility, as they cannot undergo hormonal stimulation for oocyte retrieval.

Experimental Status

Today, ovarian tissue cryopreservation and transplantation are still regarded as experimental, though successful pregnancies have been achieved following transplantation of cryopreserved tissue.

Future Directions

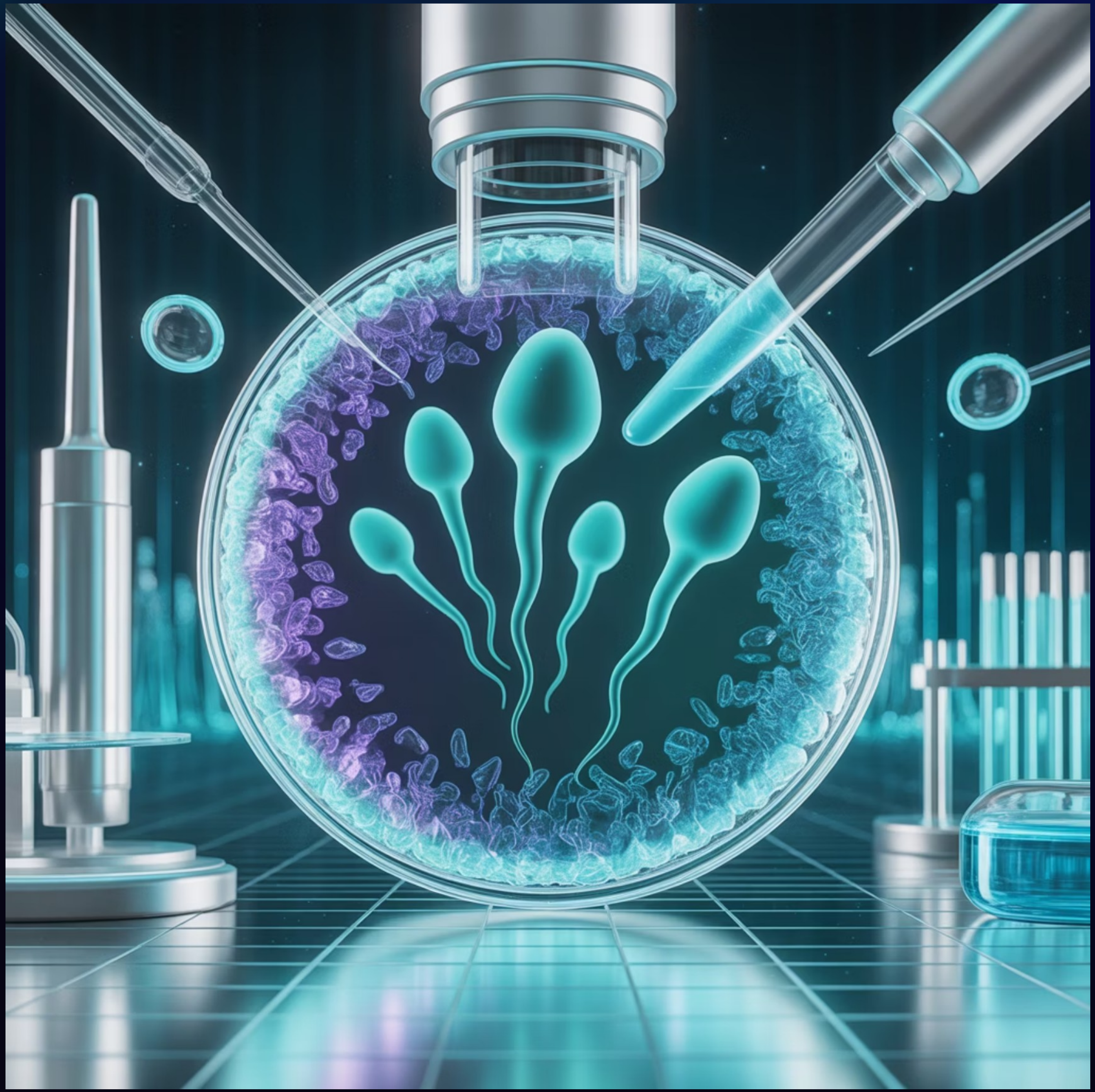
Whole ovary perfusion and vitrification are promising technologies for future clinical application, potentially offering more complete preservation of ovarian function.

There is agreement in the fertility community that this technology should not be offered to patients with benign conditions or for the purpose of delaying childbearing, as embryo and oocyte vitrification are more efficient and effective approaches.

Human Sperm Vitrification

In line with Luyet's original assertions, small cells with little intracellular water, such as sperm, can survive kinetic vitrification in the absence of permeating CPAs under rapid cooling conditions.

Evgenia and Vladimir Isachenko and their colleagues in Cologne, Germany first reported the successful cryopreservation of human sperm without cryoprotectants in 2002. Using a 0.5 M sucrose vitrification solution, these investigators developed an effective kinetic vitrification system.



These efforts were particularly well suited for the cryopreservation of low numbers of sperm

Quality Control in Vitrification

Technical Factors

Pre-vitrification organization, labeling, cryodilution, aseptic technique, container loading and sealing/protection all require standardization to ensure consistent results.

Recovery Concerns

Recovery rates should not be minimized, as they represent a potential design flaw to a device and create a serious liability risk. Published reports often hide recovery failures in their overall survival rates.

Training Importance

Training and experience are critical to reducing technical variation and ensuring reliable consistent outcomes. "Technical signature" refers to the variation in results based on operator experience.

An ideal vitrification device and method should allow for a repeatable volume of vitrification solution, containing embryo/ova, to be loaded simply in a time-sensitive, reliable, controlled manner, devoid of air bubbles. The goal is to eliminate technical variation, while optimizing 100% recovery and high survival rates.

Advantages of Vitrification

Today, the advantages of vitrification appear to significantly outweigh any potential pitfalls. Under well-controlled vitrification conditions, there should be no damaging ice crystal formation to cause osmotic, physical or physiological disruption of cellular function.

- Procedures are performed simply, reliably and rapidly
- Relatively brief exposures to concentrated, biosafe CPAs
- No need for expensive programmable freezers
- Higher survival rates compared to slow freezing
- Preservation of cellular integrity and function

Vitrification has made a lasting impact in the IVF industry over the past decade,

The Future of Vitrification

2

Advanced Research

Continued experimentation to further understand membrane functionality, the role of extracellular stabilizing additives, and ice blocking agents.

Improved Solutions

Development of optimized cryoprotectant formulations with reduced toxicity and enhanced protective properties.

Standardization

Quality management improvements aimed to reduce technical variation and enhance procedural consistency and repeatability.

Expanded Applications

Extension to whole organ preservation and other tissue types beyond reproductive cells.

The future of cellular viability is infinite in the wondrous world of metastable glass formation and the controlled elimination of recrystallization events, while maintaining normal physiological processes. As vitrification technology continues to evolve, it promises to further revolutionize reproductive medicine and tissue preservation.