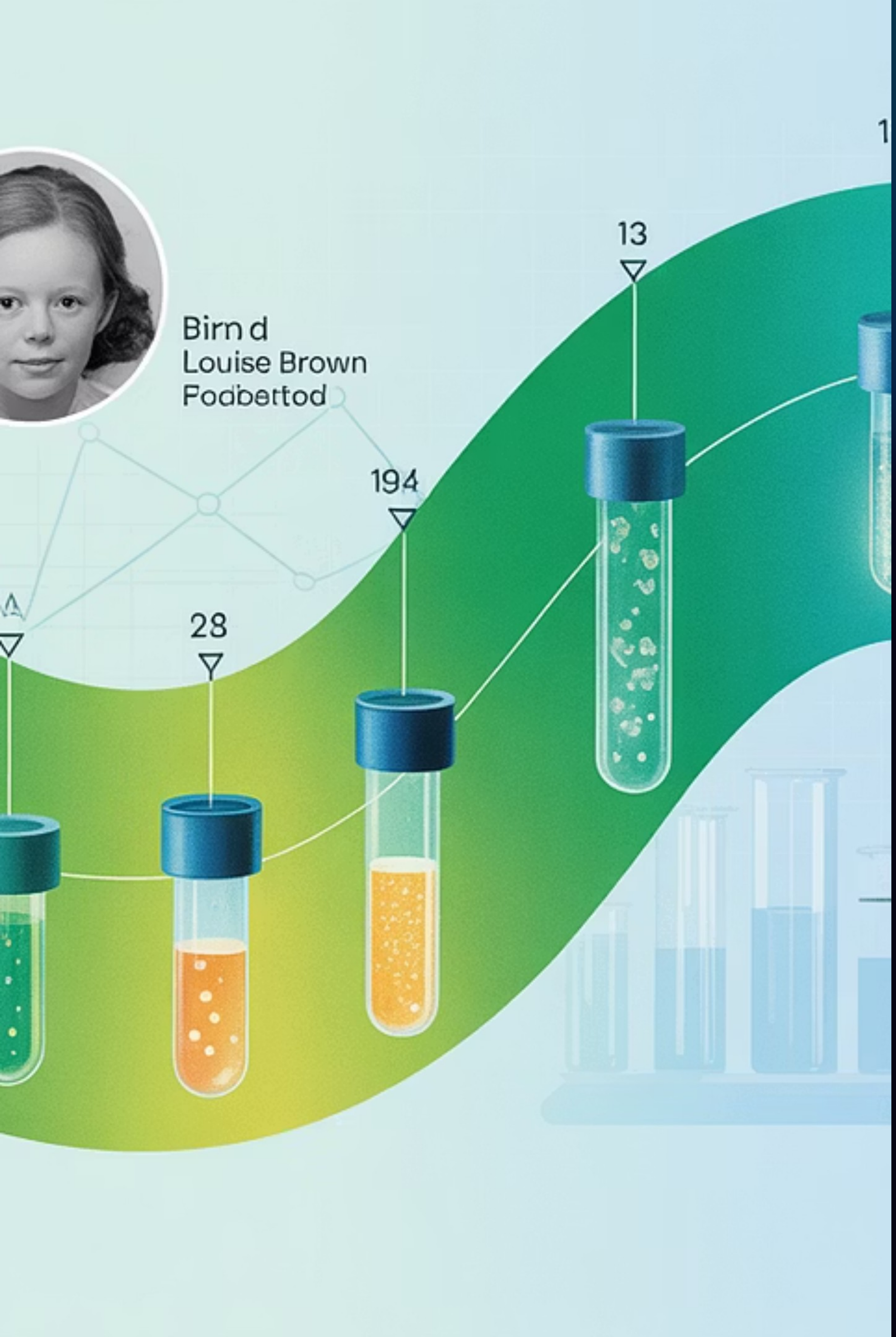
Cryopreservation of Human Embryos and Gametes: Best Practices in Storage and Laboratory Safety

Cryopreservation of human embryos and gametes is now a core component of assisted reproductive technologies (ART), occupying a place alongside key techniques such as intracytoplasmic sperm injection (ICSI) and preimplantation genetic testing. Although decades of research and clinical refinement have been devoted to the optimal freezing and warming of gametes and embryos, less attention has traditionally been paid to the protocols and systems surrounding their storage. Recent catastrophic losses at two reputable fertility laboratories have made it abundantly clear that the storage of cryopreserved reproductive tissues requires just as much vigilance, scrutiny, and rigor as the procedures to vitrify or thaw them.

The Custodians of Future Families

As embryologists, we are custodians of our patients' future family-building hopes. The loss of cryopreserved eggs and embryos is not simply a technical failure—it is an emotional and ethical crisis. These losses spurred urgent introspection across the field. Clinics everywhere asked themselves and their patients: "Could this happen to us?"

In response, thought leaders like Dr. Schiewe and his colleagues have called attention to the gap between common and best practices in cryostorage management. They highlight the consequences of complacency and urge IVF laboratories to adopt comprehensive total quality management (TQM) programs. This lesson outlines those practices and standards, integrating current regulatory requirements and expert recommendations to help IVF labs protect cryogenic inventories and prevent future incidents.



Cryopreservation in the Context of Evolving ART

When the world welcomed Louise Brown, the first child born through in vitro fertilization (IVF), in 1978, the landscape of reproductive medicine changed forever. Initially, embryo cryopreservation was an adjunctive tool—used to preserve surplus embryos after a fresh transfer. But the field evolved rapidly. Advances in embryo culture systems enabled blastocyst development, opening the door to trophectoderm biopsy and genetic screening. Vitrification techniques dramatically improved post-thaw survival rates, enabling cryo-all cycles that delay embryo transfer until a more physiologic, non-stimulated uterine environment is available.

Evolution of Embryo Culture and Transfer

1978: First IVF Birth

Louise Brown's birth marks the beginning of a new era in reproductive medicine

Early IVF Era

Embryo cryopreservation used primarily as an adjunctive tool for surplus embryos

Advanced Culture Systems

Improvements enabling blastocyst development and trophectoderm biopsy

Vitrification Era

Dramatically improved post-thaw survival rates enabling cryo-all cycles

Present Day

Single embryo transfer (SET) becoming standard practice

Improvements in incubator design, culture media, and reduced oxygen atmospheres have further increased embryo viability. With increased success rates came a shift in clinical strategy: from multiple embryo transfers to single embryo transfer (SET), decreasing the risks of multiple gestations. These developments led to a significant rise in cryopreserved embryo and oocyte volumes.

The Central Role of Cryopreservation Today

Today, cryopreservation is no longer a backup—it is central to many ART treatment strategies. The rise in preimplantation genetic testing (PGT), egg freezing for elective fertility preservation, and deferred embryo transfer cycles have transformed the embryology lab into a high-volume biorepository. Consequently, embryologists now manage both a clinical laboratory and a long-term cryostorage facility.

Total Quality Management (TQM) in Cryopreservation

The cornerstone of safe cryopreservation is a robust TQM program—one that begins before a single drop of liquid nitrogen (LN_2) is introduced.

Written Protocols

Regularly reviewed and updated standard operating procedures

Staff Training

Comprehensive initial training and continuing education

Systematic Record-Keeping

Detailed documentation of all procedures and checks

Redundant Safety Systems

Multiple layers of protection against system failures

Regular Audits

Scheduled equipment checks and protocol compliance reviews

Informed Consent: The Cornerstone of Ethical Cryopreservation

Before initiating cryopreservation procedures, fertility clinics must secure comprehensive informed consent, ensuring patients fully comprehend the long-term implications of storing their reproductive tissue. Properly structured, legally sound consent documents serve dual purposes: they empower patients to make truly informed decisions about their genetic material while establishing clear parameters for future disposition. This documentation is particularly crucial in preventing the abandonment of embryos—an increasingly common challenge that creates both ethical dilemmas and practical storage issues for fertility centers.

Staff Training and Personal Protective Equipment

Thorough Training

Personnel must be thoroughly trained in the safe handling of LN_2, cryodevices, and cryoprotective agents.

Required PPE

Staff should use face masks, cryogenic gloves, goggles, and lab coats when handling liquid nitrogen.

Proper Ventilation

Tanks must be stored in well-ventilated areas to prevent hypoxia due to nitrogen off-gassing.

Safety Documentation

A Safety Data Sheet must be available, and signage should clearly indicate cryogenic hazards.

Sample Identification and Labeling

Every cryopreserved specimen must be labeled with at least two unique patient identifiers. Clear, unambiguous labeling ensures sample traceability and ownership, reducing the risk of mix-ups or accidental discard.

- Use permanent, cryogenic-resistant labels
- Include at least two unique patient identifiers
- Verify labels during all handling procedures

Storage Tank Maintenance and Monitoring

Cryostorage tanks must be regularly inspected for structural integrity. Warning signs such as condensation, frost, pooling water, rust, or rapid nitrogen consumption may indicate vacuum failure. Automatic LN_2 filling systems should also be checked routinely to confirm they are functioning correctly. Even well-maintained systems can fail, so redundancy is critical.

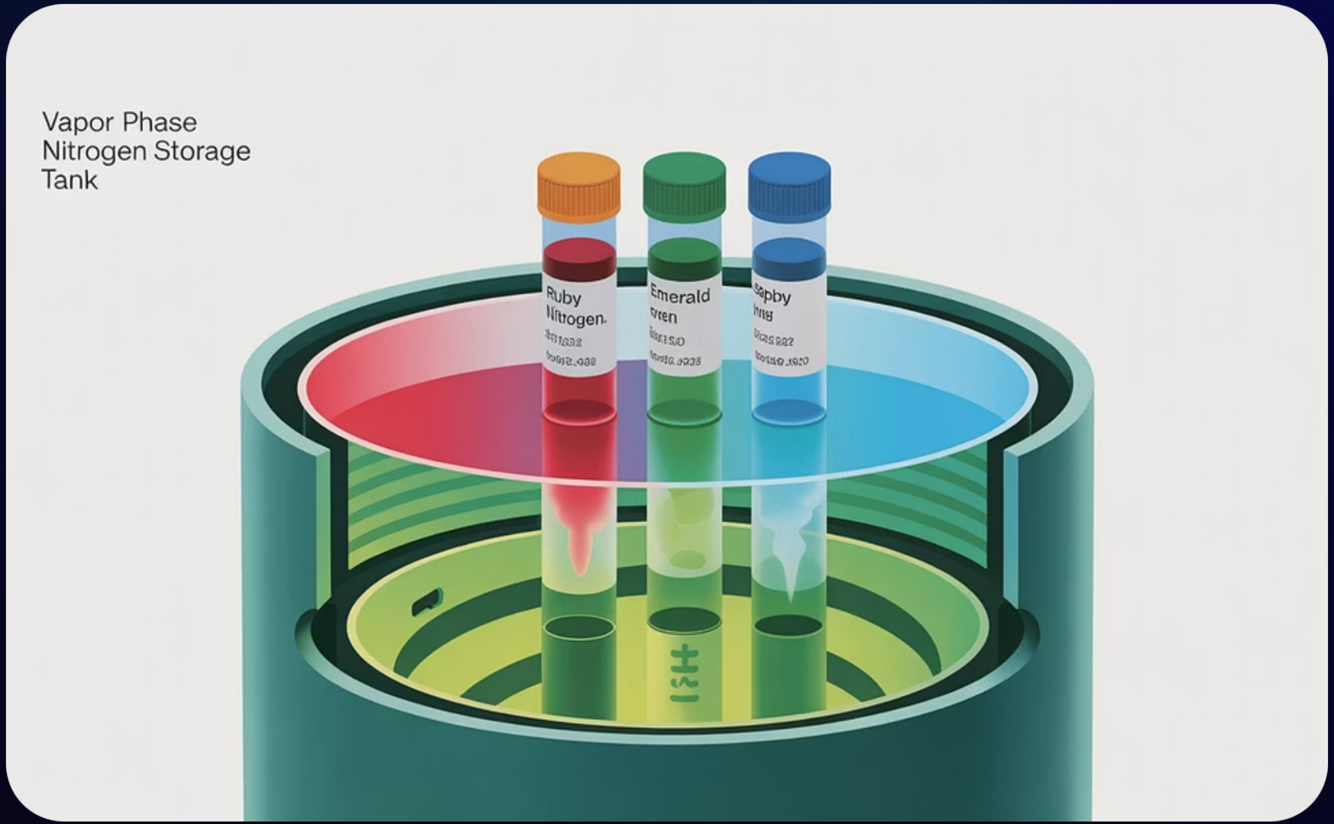
CAP Requirements for IVF Laboratories

1. Monitor and record LN_2 levels or temperature at least three times per week, or use continuous monitoring systems with alarms
2. Keep documentation of all visual inspections, alarm tests, and LN_2 replenishment
3. Have onsite reserves of LN_2 sufficient for emergency refills or tank transfers
4. Each tank should have a defined critical LN_2 level
5. A backup tank (monitored and maintained with the same rigor as active tanks) must be available to receive samples in case of a primary tank failure

Cryostorage Environment

Cryostorage tanks should be stored in secure, access-controlled areas visible to staff, ideally within or adjacent to the main laboratory. Tanks stored in isolated or poorly trafficked rooms are more likely to be neglected. Storage rooms should be equipped with oxygen monitors with both visual and audible alarms, mounted 4–6 feet off the ground to alert staff in the event of oxygen displacement by nitrogen gas.

Liquid vs. Vapor Phase Storage



Liquid Phase Storage

Maintains samples at −196°C and provides a longer buffer period during system failure.

Vapor Phase Storage

Often used for virus-positive samples or long-distance shipping. Maintains temperatures at −150°C or colder but can allow for a more rapid temperature rise if the system fails.

Inventory Management

Although full inventories of cryogenic tanks are not routinely performed (to avoid exposing fragile samples to warming), periodic reconciliation of storage logs with physical contents is essential. When inventories are conducted—such as after an alarm event—they must be witnessed and conducted under LN_2 to avoid inadvertent warming. Cryo-canes must be clearly labeled, and two identifiers must be confirmed by both the embryologist and the witness.

Cryostorage Tank Alarms and Emergency Response

All tanks must be connected to electronic alarm systems that detect temperature or LN_2 level anomalies.

Power Backup

Systems must operate during power outages (via UPS battery or generator)

Multiple Alert Methods

Send alerts to designated staff via phone, text, and audible alarms

Regular Testing

Alarm systems must be tested at least quarterly

Emergency Planning

Must be paired with a clear, written emergency response plan and a phone tree

Probes that detect LN_2 levels (rather than just temperature) are preferred, as tanks can maintain cold temperatures even when nearly empty due to vacuum insulation.

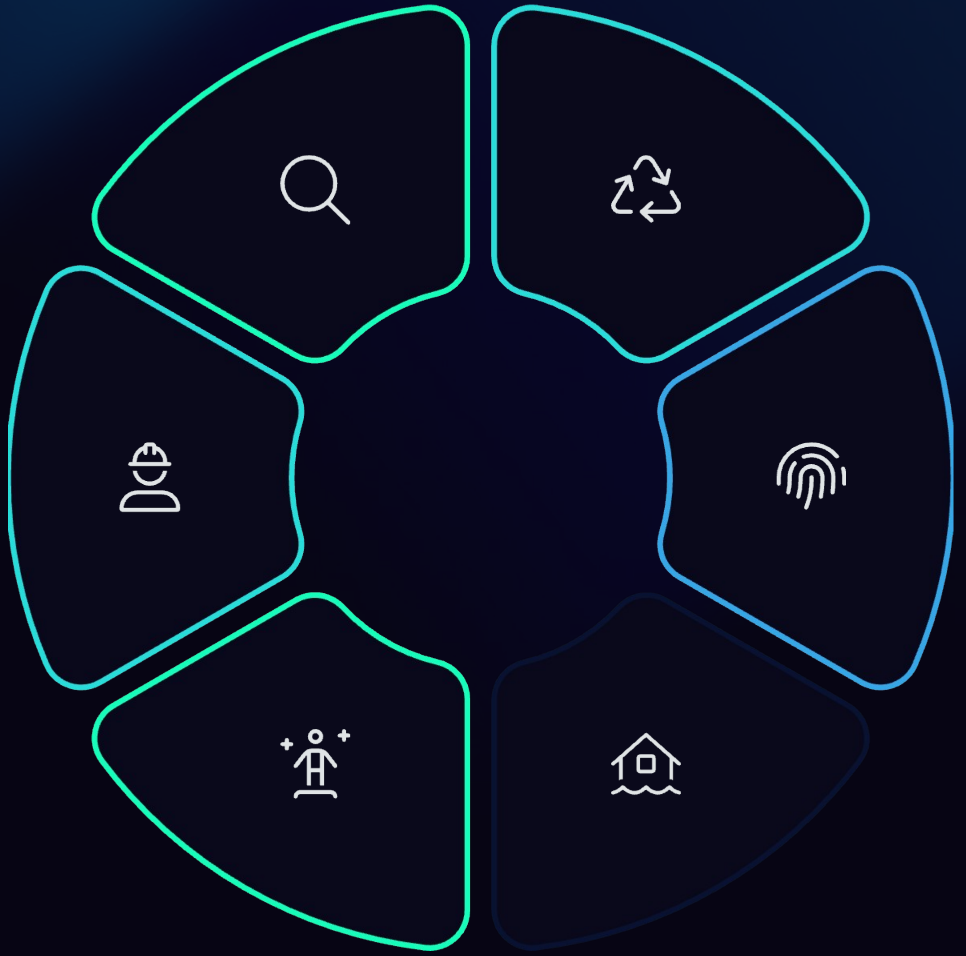
Regulatory Compliance and Standards

The CAP and The Joint Commission (TJC) set the minimum standards for cryostorage in accredited laboratories. These guidelines are also enforced by the Society for Assisted Reproductive Technology (SART) for its member programs.

- Three-times-per-week tank inspections or continuous monitoring
- Training in LN_2 safety and PPE use
- Properly ventilated storage areas
- Adequate LN_2 reserves
- Documented alarm system procedures and responses

Sources of Errors and Failures

Despite best efforts, failures can still occur. Common causes include:



Equipment

Vacuum seal failure, autofill malfunction, probe malfunction

Materials

Label failures, improper sealing or use of carriers

Safety Lapses

Frostbite, asphyxiation risks, lack of disaster planning

Human Error

Mislabeling, protocol deviation, poor inventory management

Transport Issues

Lost or mislabeled shipments, unviable samples post-thaw

Natural Disasters

Fire, flood, earthquakes

Every IVF lab must develop protocols to mitigate these risks and respond quickly if they occur.

Risk Mitigation Strategies

Preventive Maintenance

Regular scheduled inspections and maintenance of all equipment

Redundant Systems

Backup tanks, power supplies, and monitoring equipment

Staff Training

Comprehensive initial and ongoing education on protocols and emergency procedures

Documentation

Thorough record-keeping of all procedures, checks, and maintenance activities

The Human Element: Beyond Technical Protocols

The reproductive tissues stored in cryostorage tanks represent more than biological samples—they embody the dreams and commitments of patients who entrust us with their future families. As stewards of this sacred responsibility, embryologists and ART laboratories must hold themselves to the highest standards of quality and safety.

While no system is immune to failure, the conscientious application of best practices in cryopreservation—paired with vigilance, redundancy, and transparency—can minimize risk and ensure that patient samples are treated with the care and respect they deserve.

Conclusion: Honoring the Promise of Reproductive Medicine

> By treating cryopreservation as a specialty in its own right, and by embracing a culture of continuous improvement and accountability, we honor the science, the patients, and the promise of reproductive medicine.

As the field of assisted reproductive technology continues to advance, our commitment to safeguarding the precious materials entrusted to our care must remain unwavering. Through rigorous protocols, continuous education, and an ethical framework that places patient welfare at its center, we can ensure that cryopreservation remains a cornerstone of successful fertility treatment for generations to come.