Vitrification

Say Goodbye to Slow-Freezing

IrvineScientific®

Grow With Us™

Benefits of Vitrification Vs. Slow-Freezing

Better Pregnancy Rates with Fewer Embryos Transferred

Multiple pregnancies are the greatest complication resulting from IVF treatment, and for many patients transfer of a single embryo is now recommended. However, this practice risks an overall lower pregnancy rate per cycle, when compared to cycles where multiple embryos are used. But the introduction of vitrification to clinical practice has the potential to allow for **very reliable recovery of cryopreserved embryos,** giving practitioners and patients more confidence to transfer fewer embryos in the fresh cycle.

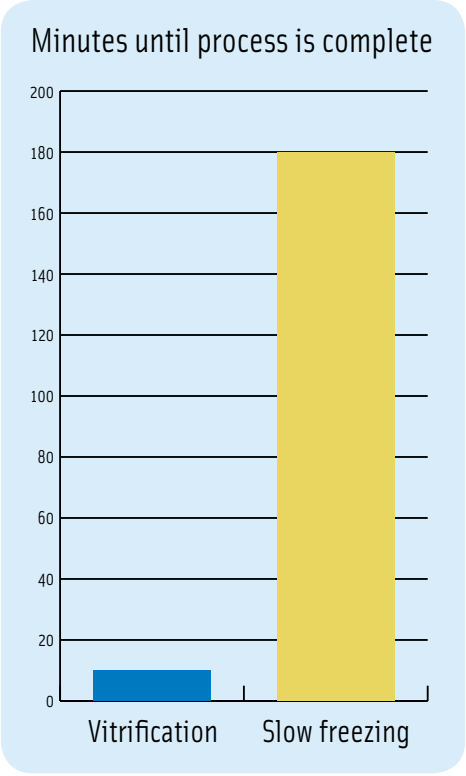
If pregnancy and implantation rates with preserved embryos can be shown to be similar to those with fresh embryos, the need to transfer multiple embryos in any cycle will be greatly reduced. Data confirms that **excellent pregnancy rates can be achieved with vitrified blastocysts** in patients of all ages in frozen embryo transfer cycles, thus allowing clinics to move to elective single embryo transfer.[1]

Decreased Chilling Injury

Compared to slow-freezing, the rate oocytes and embryos pass through the dangerous temperature zones (15 to -5°C) with vitrification are extremely high, and the very short exposure to dangerous temperatures **radically decreases the injury of the sensitive structures.**[2]

Time is Valuable

Instead of spending several hours slow-freezing, **the vitrification process can be completed in 10 minutes,** allowing more time to perform other critical lab tasks.



Reasons to Choose Vitrification

"WHEN (not if!) IVF programs overcome fear of the unknown, and take on the challenge of a significant learning curve with vitrification, THEN vitrification will become the clinical standard for human oocyte and embryo cryopreservation."

Juergen Liebermann, PhD
Laboratory Director
Fertility Center of Illinois
Chicago, IL

"Here at HRC-Laguna Hills, we switched to vitrification (Irvine Scientific's Vit Kit®-Freeze/Thaw and CryoTips) as of March of 2007. Since then we have seen a significant increase in both our embryo survival rates (+35 percentage points) and our clinical pregnancy rates (+23 percentage points). Biopsied blastocysts tolerate vitrification better than slow freeze cryopreservation."

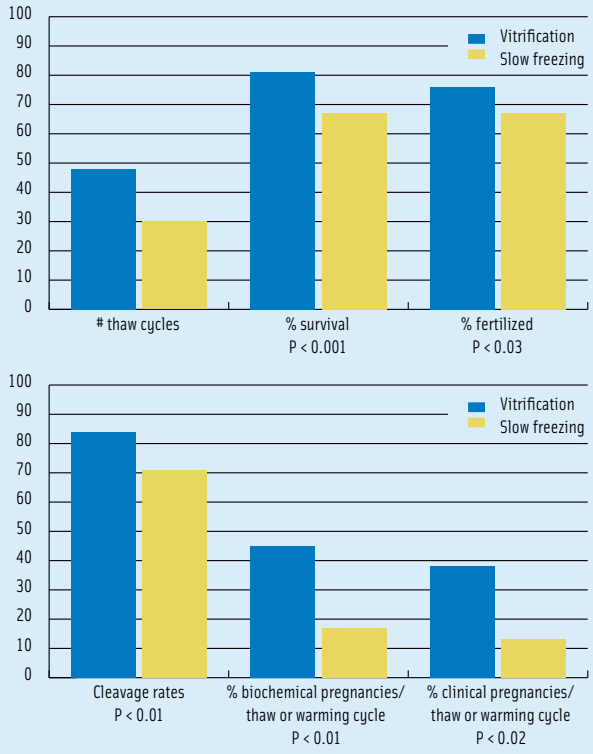
Charlotte Khoury
Scientific Director
Huntington Reproductive Center
Laguna Hills, CA

Vitrification is Superior to Slow-Freezing

Significantly Improved Pregnancy Rates with Oocyte Vitrification

A study published in *Fertility and Sterility* compared the cryopreservation of mature human oocytes by either slow-rate freezing or vitrification (Irvine Scientific Vit Kits with CryoTips) to determine which method is the most efficient at establishing a pregnancy.[3]

Figure 1. Oocyte Survival and Functionality Following Cryopreservation by Slow-Rate Freezing/Thawing or Vitrification/Warming (Smith et al., 2010)



Vitrification/warming resulted in significantly higher cryosurvival, fertilization, embryo cleavage and development and clinical pregnancy rates in comparison to slow-rate freezing/thawing.

Significantly Higher Survival Rates with Oocyte Vitrification

The outcome of a study on oocyte vitrification, conducted at the Cryobiology Unit at Instituto Valenciano de Infertilidad (IVI), was published in *RBMonline* to evaluate the influence of four different cyropreservation protocols on freshly collected human oocytes.[4]

Table 1. Survival of Oocytes After Cryopreservation (Cobo et al., 2008)

Cryopreservation protocol	No. of oocytes	No. surviving (%)
Slow freezing + 0.2 mol/l sucrose	36	23 (63.8)[a]
Slow freezing + 0.3 mol/l sucrose	34	25 (73.5)[b]
Slow freezing + choline replacement	36	20 (74.1)
Vitrification by CryoTip® method	27	20 (86.9)[a,b]
Control (fresh oocytes)	34	34
Total	167	122

a,b Values with the same superscript letter are significantly different (P<0.05).

Vitrification with the CryoTip® showed that survival for vitrified oocytes was significantly higher than that observed for slow-freezing with 0.2 and 0.3 mol/l sucrose ($p<0.05$). This suggests that vitrification with the CryoTip® is superior to slow-freezing at preserving the oocytes' ability to develop into competent embryos.

Visual Demonstration of Post-Vitrification Survival

Improved Embryo Survival with Vitrification

By avoiding intracellular ice formation associated with slow-freezing, vitrification minimizes embryo damage.

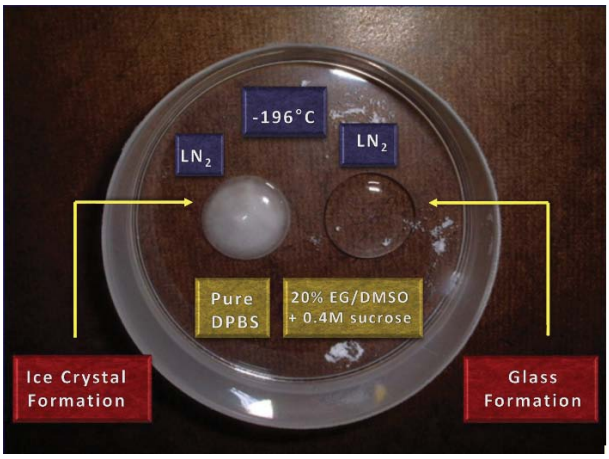
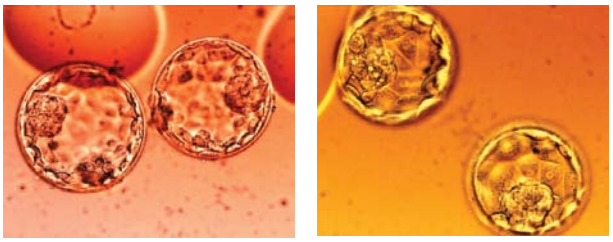


Photo Courtesy of Juergen Liebermann, PhD, Fertility Center of Illinois.

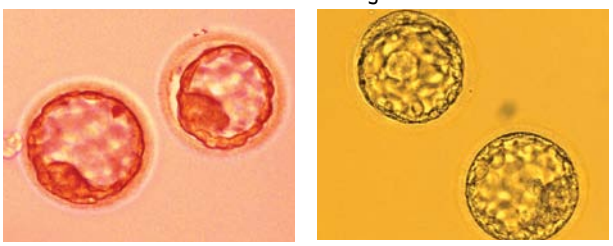
Embryo Quality Appears Unchanged Post-Vitrification

Thawed blastocysts that have undergone vitrification look identical to fresh blastocysts, indicating that human embryos tolerate vitrification well.

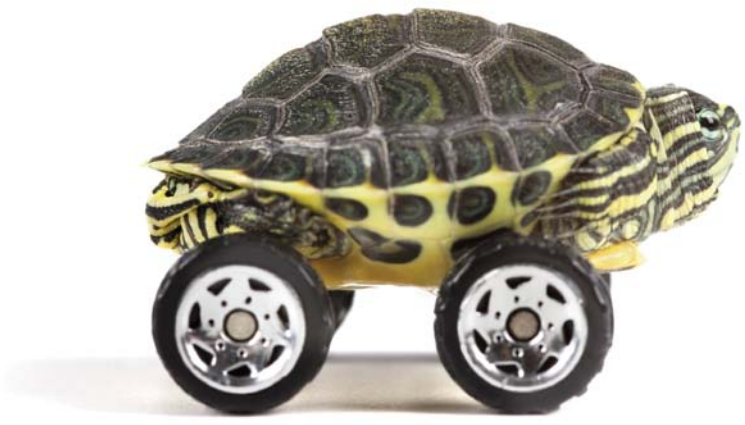
Fresh Human Blastocysts



Vitrified/Warmed Human Blastocysts



Photos Courtesy of Juergen Liebermann, PhD, Fertility Center of Illinois.



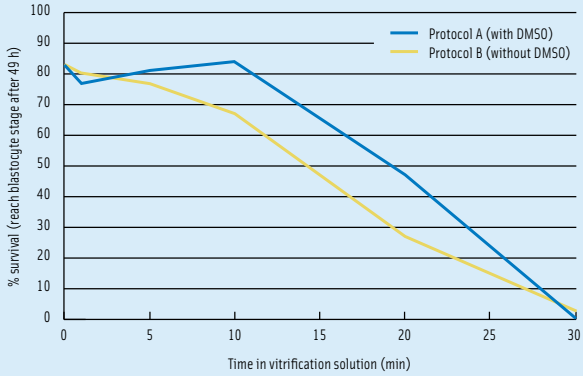
Selecting Cryoprotectants

DMSO-Containing Vitrification Solutions

Ice-crystal formation that is associated with slow-freezing can be lethal to oocytes and embryos. Cryoprotectants, both permeating and non-permeating, are used to reduce ice-crystal formation by dehydrating the cells before the vitrification process. All cryoprotectants are potentially hazardous. The hazards associated with cryoprotectants can be greatly minimized by using a combination of cryoprotectants at reduced concentrations, limiting the time of exposure, and using a proven method that allows for rapid vitrification.

Dimethylsulfoxide (DMSO) and ethylene glycol (EG) are the most widely used permeating cryoprotectants in vitrification solutions for human oocytes and embryos, while sucrose is the most commonly used non-permeating cryoprotectant.

Figure 2. Comparison of Protocols A (with DMSO) and B (without DMSO) in Mouse (Kartberg et al., 2008)



A study investigating the chemical injury associated with vitrification showed that the DMSO-containing vitrification solution leads to significantly less chemical injury upon prolonged exposure compared with the DMSO-free vitrification solution, possibly due to the fast penetrating characteristics of DMSO.[5]

An Optimized Vitrification System

Irvine Scientific Vit Kits contain a combination of two permeating cryoprotectants (DMSO and EG) and sucrose as the non-permeating cryoprotectant. These solutions provide optimal concentrations with a proven protocol that requires short exposure times to solutions at favorable temperatures, consequently enabling rapid vitrification when plunged into liquid nitrogen without the formation of ice crystals yielding higher survival and pregnancy rates than those for slow-freezing.

Being a pioneer that has made vitrification commercially available to IVF clinics worldwide since 2005, we've had the privilege of working closely with hundreds of embryologists across the world as they adopted vitrification into their practice. As a result, we have learned a great deal from our customers about some of the practical challenges and solutions that go along with adopting a new technique such as vitrification. This unique insight gives us the ability to support your transition and continued success with great confidence that only comes with years of experience. Our skilled technical application scientists and customer service representatives are an extension of our products that are at your disposal as you, too, say goodbye to slow-freezing.

Advantages of Irvine Scientific Vitrification Kits

Convenient Kits

- *Unique packaging identifying each component resulting in quick identification and efficient use*
- *Available with just media to support any cryodevice*
- *Available with a cryodevice: CryoTip® or HSV Straw*

Regulatory Compliant

- *CE marked media for gametes and embryos*
- *FDA cleared media for embryos of all stages*
- *CryoTip® is CE marked and FDA cleared*
- *HSV Straw is CE marked*

Kits Provide Multiple Applications

- *Can be used on more than one patient*
- *Less waste and more cost-effective than other kits that are single patient, single usage*

Eight Week Shelf-Life After Opening

- *Longer shelf-life than other commercially available kits*
- *Save time and money by ordering less often*

Simple, Straight-Forward Protocols

- *Easy to learn and perform*
- *Removes uncertainty when training*

Outstanding Customer Support

- *Knowledgeable staff on hand to support your technical needs*
- *Available to assist you with accurate and on-time order processing*
- *Committed professionals who strive to establish lasting relationships*

Irvine Scientific Media for Vitrification

Media Composition

The composition of these solutions is based upon published methods and designed in collaboration with Dr. Masashige Kuwayama utilizing a combination of EG and DMSO as the permeating cryoprotectants. The basal medium for these solutions is Modified M199 (containing 21 mM HEPES buffer) supplemented with 20% DSS (HSA and Dextran, containing 10 mg/mL protein) and 35 µg/mL gentamicin as the antibiotic.

Solution	DMSO (v/v)	EG (v/v)	Sucrose
Equilibration Solution (ES)	7.5%	7.5%	0
Vitrification Solution (VS)	15%	15%	0.5M
Thawing Solution (TS)	0%	0%	1.0M
Dilution Solution (DS)	0%	0%	0.5M
Washing Solution (WS)	0%	0%	0

Vit Kit® Configurations

Available configurations for USA

Vit Kit® – Freeze (for embryos – 2PN through blastocyst)
Order No: 90133-DSO
Contents: ES 2x1 mL, VS 2x1 mL

Vit Kit® – Thaw (for embryos – 2PN through blastocyst)
Order No: 90137-DSO
Contents: TS 4x1 mL, DS 1x1 mL, WS 1x2 mL

Available configurations Worldwide (excluding USA)

Vit Kit® – Freeze (for oocytes and embryos of all stages)
Order No: 90133-HSV
Contents: ES 2x1 mL, VS 2x1 mL, 8 HSV Straws

Order No: 90133-SO
Contents: ES 2x1 mL, VS 2x1 mL

Vit Kit® – Thaw (for oocytes and embryos of all stages)
Order No: 90137-SO
Contents: TS 4x1 mL, DS 1x1 mL, WS 1x2 mL

Devices for Vitrification

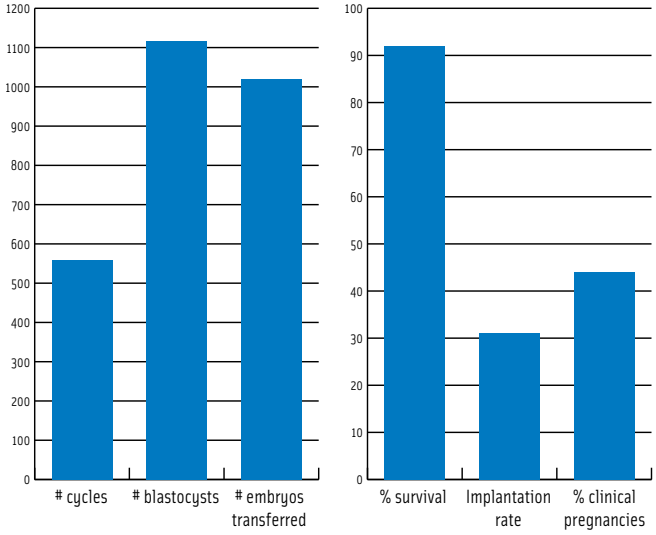
Irvine Scientific provides flexibility by offering two closed system devices. Clinical data on blastocyst stage embryos with Irvine Scientific media is presented for both the CryoTip®[6] and the HSV Straw.[7]

CryoTip®

The CryoTip® is a finely pulled straw designed for holding gametes or embryos during cryopreservation procedures and long-term frozen storage. This closed-system device is widely used by fertility clinics around the world.

- Metal sleeve to protect the tip and specimens during storage
- 510k cleared
- CE marked
- Closed system

Figure 3. Clinical Data on Vitrification with Irvine Scientific Media and the CryoTip® (Conaghan et al., 2010)

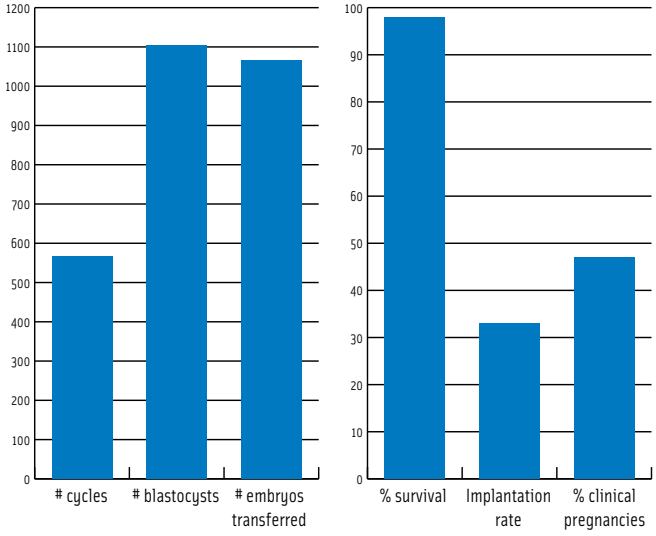


HSV Straw

The HSV Straw is a completely closed system when thermally sealed using the SYMS sealer resulting in complete containment of vitrified specimens.

- High quality ionomeric resin straw
- Available in six different colors to aid in traceability
- CE marked
- Closed system

Figure 4. Clinical Data on Vitrification with Irvine Scientific Media and the HSV Straw (Liebermann el al., 2010)



Quality Testing

Each lot receives a complete laboratory evaluation including mouse embryo testing, endotoxin level, pH, osmolality and sterility testing. All results are provided in a lot-specific Certificate of Analysis.

Irvine Scientific's commitment to excellence is demonstrated by its products' performance and adherence to the industry's highest quality standards. Irvine Scientific was the first ART manufacturing company in the USA to receive ISO 13485:2003 quality systems certification, the rigorous international quality assurance standard designed specifically for Medical Devices.

Products manufactured by Irvine Scientific are produced in accordance with the Guidelines for Manufacture of In Vitro Diagnostic Products and the Good Manufacturing Practices (GMPs) for Medical Devices.

References

1. Conaghan et al., (2010), Human blastocyst Vitrification and Warming: 3 Years Experience Using the CryoTip®
2. Tucker M, Liebermann J, (2007), Vitrification in Assisted Reproduction. United Kingdom. Informa Healthcare
3. Smith et al., (2010), Prospective randomized comparison of human oocytes cryopreservation with slow-rate freezing or vitrification, Fertility and Sterility, Feb 18 2010
4. Cobo et al., (2008), Effect of different cryopreservation protocols on the metaphase II spindle in human oocytes, Reproductive BioMedicine Online, Volume 17, 16 July
5. Kartberg A-J et al., (2008), Vitrification with DMSO protects embryo membrane integrity better than solution without DMSO, Reproductive BioMedicine Online, Volume 17, 3 September
6. Data on file.
7. Data on file.

North America Headquarters
Irvine Scientific
2511 Daimler Street, Santa Ana, CA 92705
USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
www.irvinesci.com

Europe Headquarters
Irvine Scientific
Unit 31, Newton Business Center, Block D
Newtownmountkennedy
County Wicklow
Ireland
Phone: +353 1 281 99 20
Fax: +353 1 281 99 28

Asia Headquarters
Irvine Scientific
3-17-35 Niizo-Minami
Toda-Shi
335-0026 Saitama
Japan

IrvineScientific®
Grow With Us™

2012 Irvine Scientific P/N 10034R Rev.11