Optimizing Vitrification Outcomes using UltraRapid Warm Blast

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Affiliations and Disclosures

Scientific Director: Embryology and Andrology Laboratories

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Committee Member:

Key Milestone in Mammalian & Human Embryo Vitrification

1983 – Rall & Fahy (U.S. Naval Medical Research Institute)

First successful **vitrification of mouse embryos** using high concentrations of cryoprotectants to avoid ice formation.

1990 – Gordts et al. (Leuven Institute for Fertility and Embryology, Belgium)

Among the first reports on **vitrification of human embryos**.

- **1997 – Kuleshova et al. (University of Adelaide, Australia)**

First **live birth following vitrification of human oocytes**.

- **1999 – Masashige Kuwayama (Kato Ladies Clinic, Japan)**

Developed the **Cryotop method**, which dramatically increased survival and success rates of vitrified oocytes and embryos.

- **2003 – Peter Nagy (Reproductive Biology Associates, USA)**

Played a key role in the **clinical implementation and optimization** of oocyte vitrification in the U.S. and internationally.

- **2005 – James Stechekci (Assure Fertility, USA)**

Early **adopter and promoter of vitrification** in clinical IVF, contributing to widespread use and training programs in the U.S.

2010s – Tyl Taylor (Ovation Fertility, USA), Juergen Liebermann (Fertility Centers of Illinois, USA)

Pioneers of Ultra-Rapid Warming Techniques which improved post-thaw viability and recovery of vitrified oocytes and embryos.

- 2024 – **Juergen Liebermann (Fertility Centers of Illinois, USA)**

first peer reviewed publication on ultra fast warming of human blastocysts in a clinical setting, standardizing the protocol.

Early Range Finding Study

- The objective of this study was to examine the potential toxicity of sucrose (Experiment 1) and of various cryoprotectants (Experiment 2) to porcine preimplantation embryos.

- Embryos placed into 2.0 M sucrose exhibited poorer development and quality than embryos at the lower 4 concentrations, which were not different from one another. (n=65)

- As the concentration of an individual cryoprotectant increased beyond 30%, embryo development decreased.
  - Embryos exposed to glycerol or propylene glycol exhibited poorer development than did embryos placed into ethylene glycol.
- Movement towards DMSO, Ethelyne Glycol.

Molar concentration	n	Quality score	Embryo development[a]
0	11	0.64 ± 0.15[b]	100%[d]
0.25	14	0.64 ± 0.13[b]	83 ± 17%[d]
0.50	13	0.77 ± 0.12[b]	100%[d]
1.00	14	0.71 ± 0.13[b]	100%[d]
2.00	13	0.08 ± 0.08[c]	17 ± 17%[e]

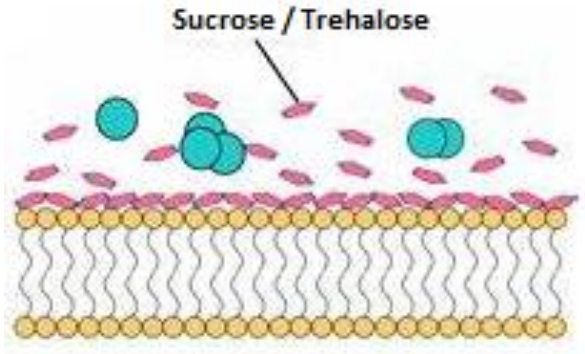
Concentration	Cryoprotectant[a]	n	Development[b]
0	none	15	93 ± 7%[cd]
10	EG	7	100%[c]
10	PG	5	100%[c]
10	GLYC	6	100%[c]
20	EG	10	50 ± 17%[efg]
20	PG	9	89 ± 11%[cd]
20	GLYC	9	78 ± 15%[cde]
30	EG	5	80 ± 20%[cde]
30	PG	5	60 ± 24%[def]
30	GLYC	4	75 ± 25%[cdef]
40	EG	8	100%[c]
40	PG	9	22 ± 15%[gh]
40	GLYC	8	0%[h]
50	EG	7	43 ± 20%[fg]
50	PG	6	0%[h]
50	GLYC	8	0%[h]

[a] EG=ethylene glycol; PG=propylene glycol; GLYC=glycerol.
[b] Excludes embryos collected at the hatched blastocyst stage.

C.R. Youngs, 1994
Iowa State University

Non-Permeating Cryoprotectant Concentrations in Warming Products

Product	NPC	NPC Concentration in TS	Osmolality (mOsm/kg)
Vitrolife Ultra RapidWarm™ Blast	Sucrose	0.25 M	527-567
Kitazato Thawing Media	Trehalose	“Proprietary”	1600-2000
Irvine Scientific Vit Kit-Warm NX	Trehalose	1 M	1500-1900

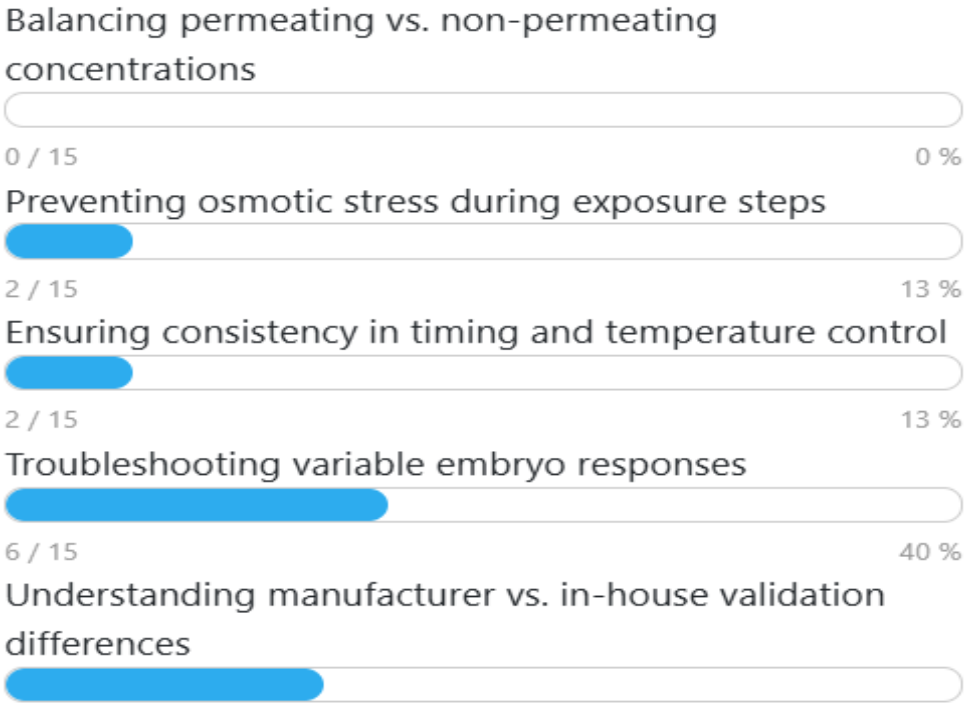


The Southwest Embryology Summit 2026

Poll question

Which aspect of cryoprotectant use do you find most challenging in daily practice?

My response



Membrane interaction with non-permeating cryoprotectant –Sugars

Osmotic Buffering

Sugars (Sucrose, Trehalose) are non permeating Cryoprotectants

Draws water out of the cells as permeating CPAs (e.g., DMSO, ethylene glycol)are removed.

Hypertonic: Prevents osmotic swelling and lysis

Major risk when CPAs are removed too rapidly

Membrane Stability

Stabilizes phospholipid bilayer

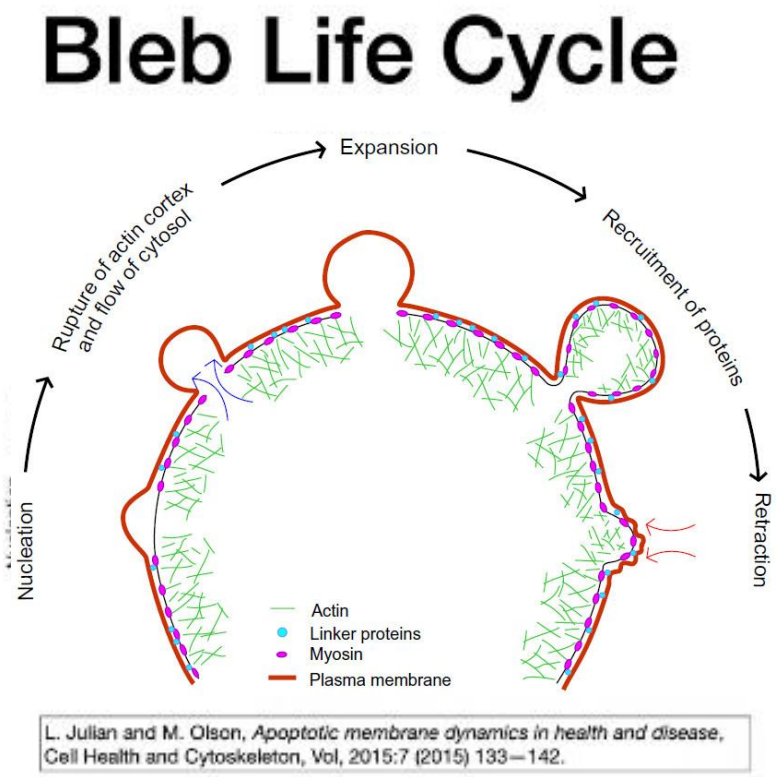
Provides hyper osmotic extracellular environment preventing abrupt water influx

Minimize intracellular Ice Formation

If water enters too quickly before CPAs exit, ice crystals can form

Sugar concentrations control the rate of exchange during rehydration.

Membrane interaction with non-permeating cryoprotectant –Sugars

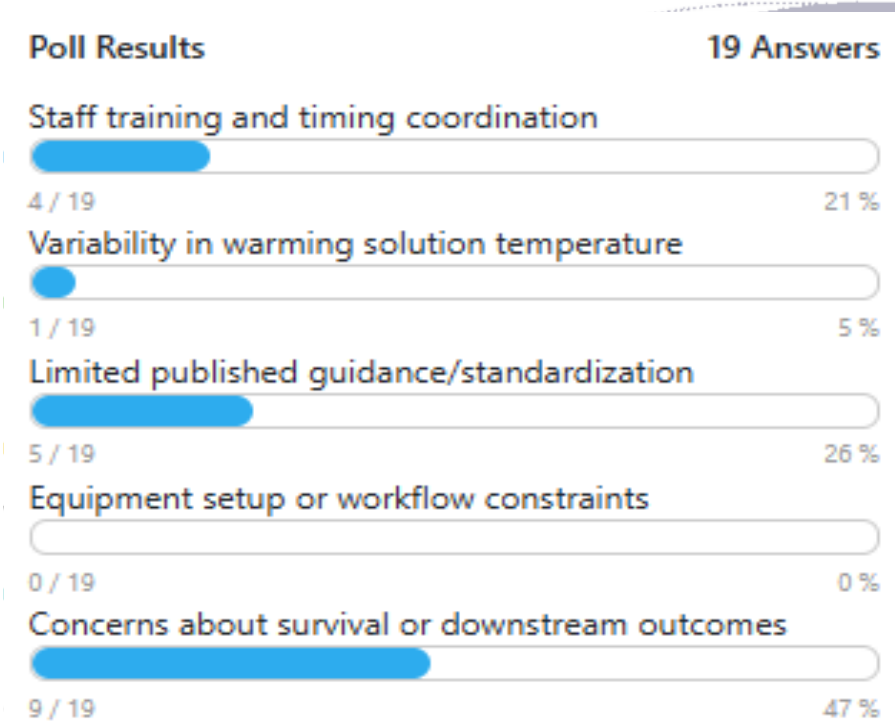


The Southwest Embryology Summit 2026

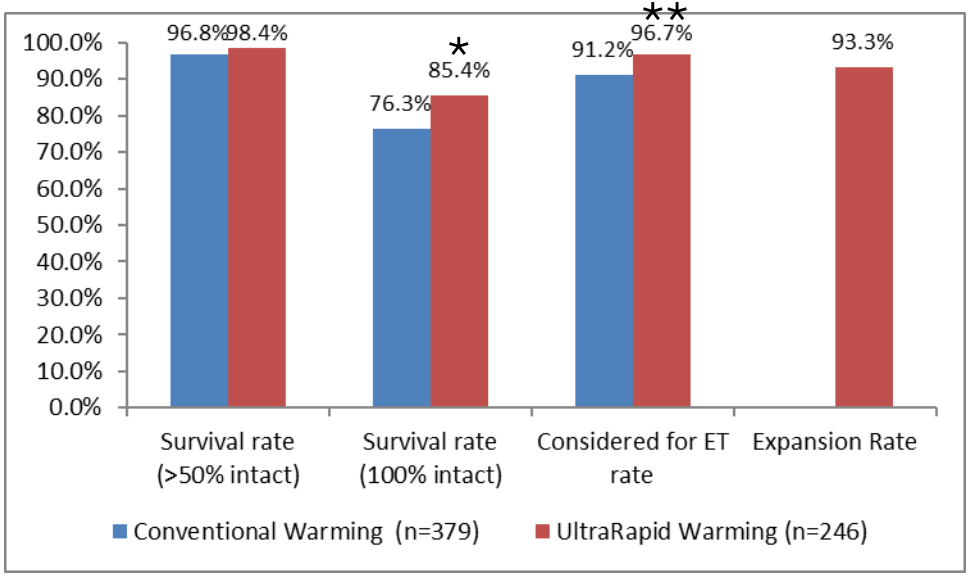
Poll question

What is the biggest barrier your lab faces when validating or implementing ultra-rapid blastocyst warming?

My response



Review of literature
Ghent University Hospital - Belgium



Findings and Impressions:

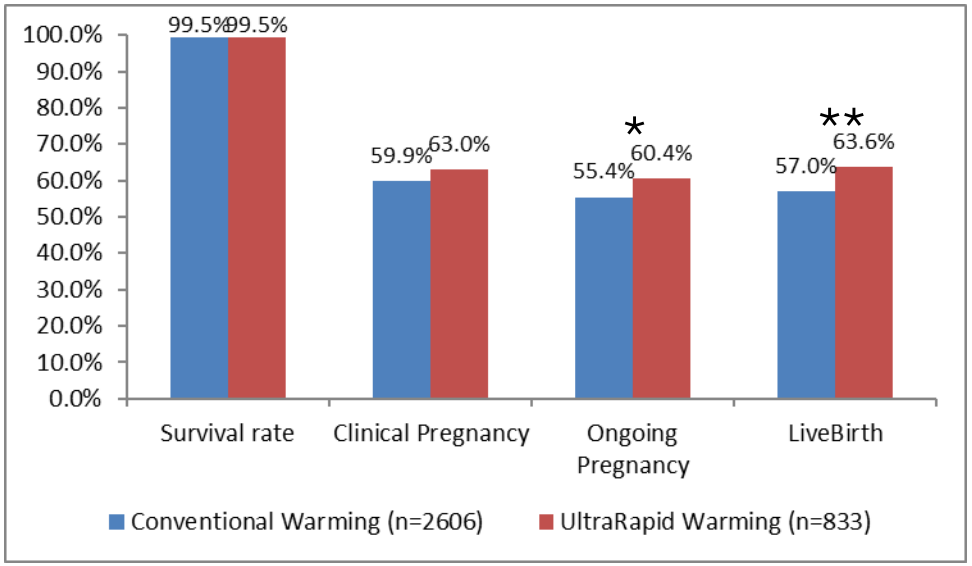
- No difference noted between biopsied and non-biopsied embryos in sub-group analysis.
- Minimizing handling steps
- Significantly shortens the procedural time.
- Improvement in the laboratory workflow and efficiency.

***Survival Rate: p=0.0076**
****Considered for ET rate: p=0.0002**

Gheselle et al. 2025
Kelly Tilleman
Irvine Vit-Kit WarmNX

First Peer-reviewed Clinical Pregnancies 2024

Fertility Centers of Illinois- USA



Liebermann et al. 2024
RBMO

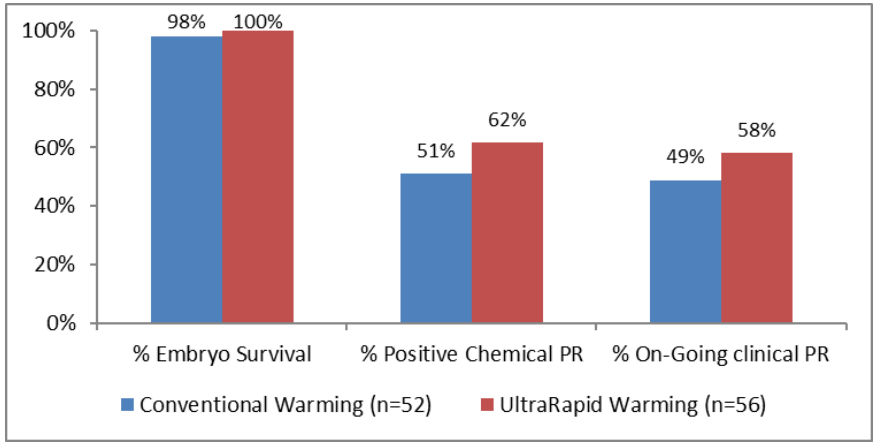
Findings and Impressions:

- Identical survival rates
- Improved Ongoing pregnancy.
- Significantly reduced miscarriage rates (4%- 1-step, 7.6% multi-step, P=0.0001)
- Blastocysts stage and biopsy status do not impact outcomes.
- Ultra Rapid method decreases thaw time without compromising survival and re-expansion.

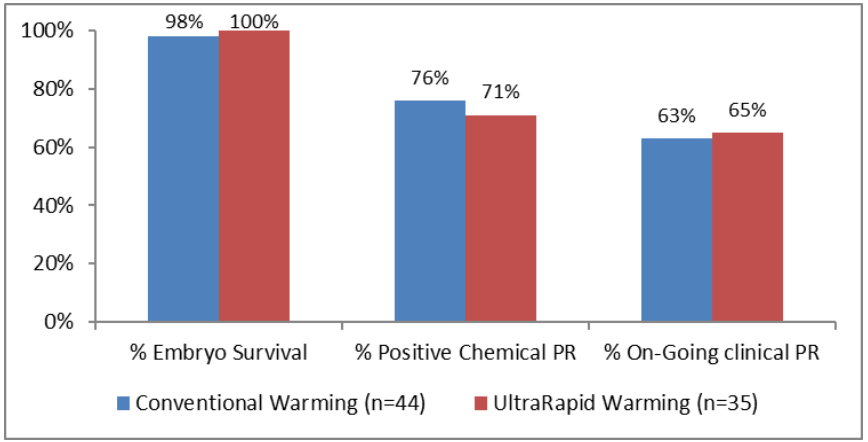
***Ongoing Pregnancy Rate: p=0.011**
****Live Birth Rate p=0.0005**

VitroLife UltraRapid Warm Fertility Specialists Network USA

IVFMD TX

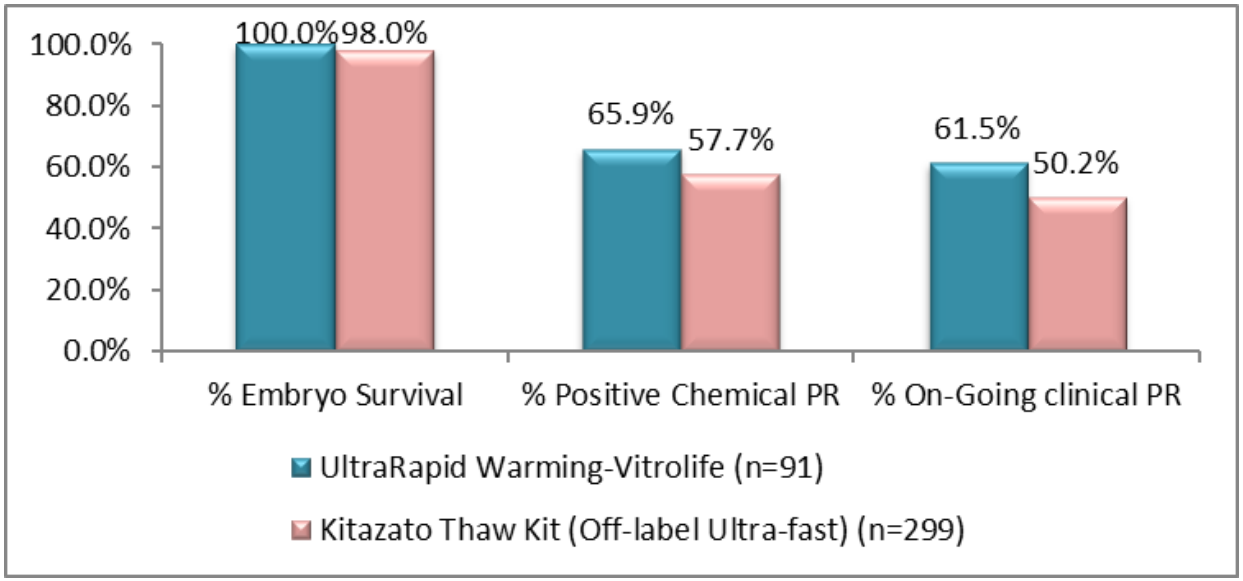


Viera Fertility Center



Ali, Sheela and Sylvie Roberge 2025 data (unpublished)

VitroLife UltraRapid Warm compared to Kitazato Thawing Media

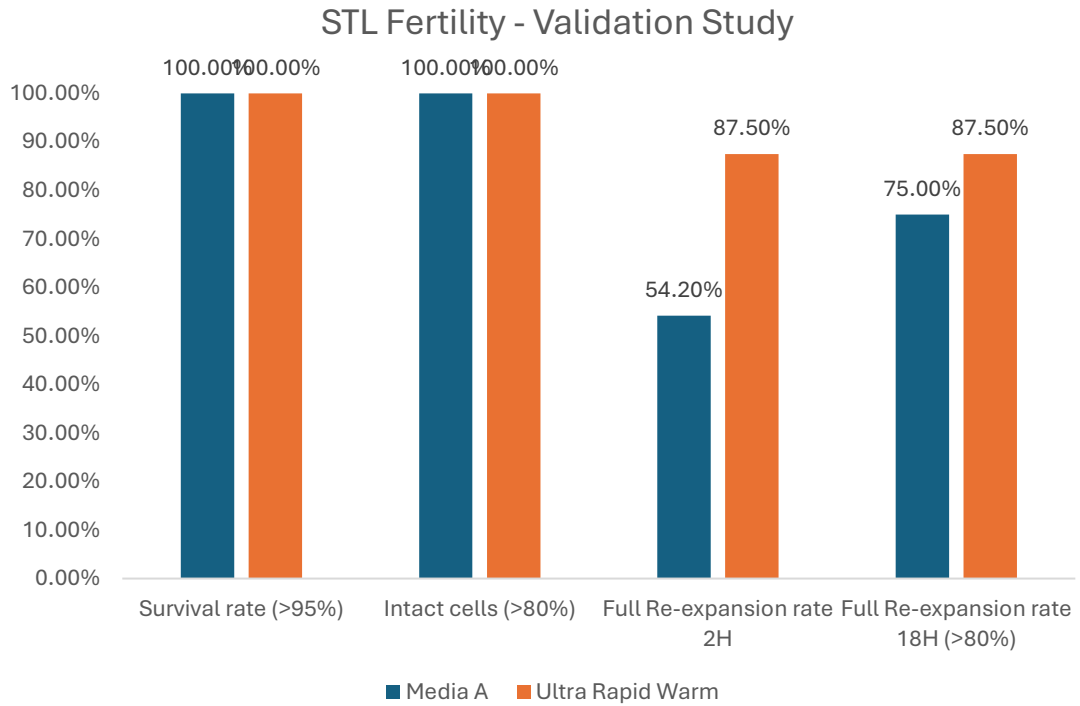


Findings and Impressions:

- Kitazato data is from single site
- Vitrolife Rapid warm data is combined data from 2 FSN sites.
- Slightly improved, but comparable embryo survival rate.
- Improved Clinical and Ongoing pregnancy rates with Vitrolife Rapid Warm

Ali, Sheela 2025 data (unpublished)

Validation Study - VitroLife UltraRapid Warm (0.25MSucrose)compared to Kitazato Thawing Media (Higher Surcrose)



	Media A	Ultra Rapid Warm
Survival rate (>95%)	24 / 24	24 / 24
Intact cells (>80%)	24 / 24	24 / 24
Full Re-expansion rate 2H	13 / 24	21 / 24
Full Re-expansion rate 18H (>80%)	18 / 24	21 / 24

Ali, Sheela 2025 data (unpublished)

Key Findings

- 0.25M Sucrose concentration improves cellular recovery post warming and treatment outcomes.
- 37ºC precise temperature control is crucial for success.
- Reduction of total time blastocysts are exposed to ambient temperatures- reducing stress and ROS creation and impact.
  - “Blebbing” (Liebermann, et al. 2024)
- Reduction of hyper osmotic conditions and TE cell loss due to damage from prolonged osmotic stress.

UltraRapid warming: Challenges and pitfalls in adoption

Training and technical precision:

- Requires precise timing and quick hands
- Unfamiliarity with SOP may cause osmotic shock or incomplete rehydration.

Post-warming processing

- Warmed embryos require thorough washing in culture media to remove TS
- Hyper osmotic solution carryover will impact embryo survival and implantation.

Equipment and Infrastructure

- Requires dedicated 37ºC workspace/incubators for warming.
- Difficult to stagger

Regulatory and Quality Documentation

- Validate internally using donated to research embryos
- Write a clear and updated SOP and perform competency assessments prior to implementation.

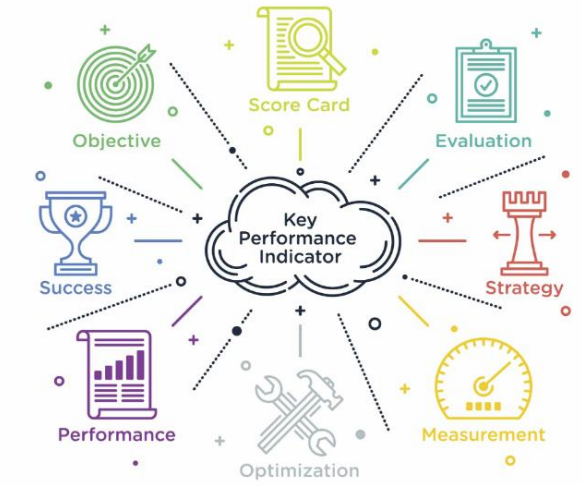
Monitor Outcomes During Transition Phase

- Early identification of variability in embryologist technique (survival, re-expansion rates)

Utilizing KPIs to Drive Warming Success

Identify core KPIs

- Survival rate
  - 2h, 4h, `18-24h post warming
  - Percentage of in-tact cells at survival
- Re-expansion rate
- Staff skill set
- Lab volume



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- Liebermann, Juergen et al. Fast and furious: pregnancy outcome with one-step rehydration in the warming protocol for human blastocysts. Reproductive BioMedicine Online, Volume 48, Issue 4, 103731
- Certificates of Analysis
  - Vitrolife
  - Kitazato
  - FujiFilm Irvine Scientific