INTERNATIONAL FEDERATION OF FERTILITY SOCIETIES

Cryopreservation – Laboratory aspects

IFFS Embryology Workshop
TARTU, ESTONIA
21-23 September 2018

This presentation is made possible, in part, with the generous non-restricted educational grant support from *our sponsor for this workshop:*

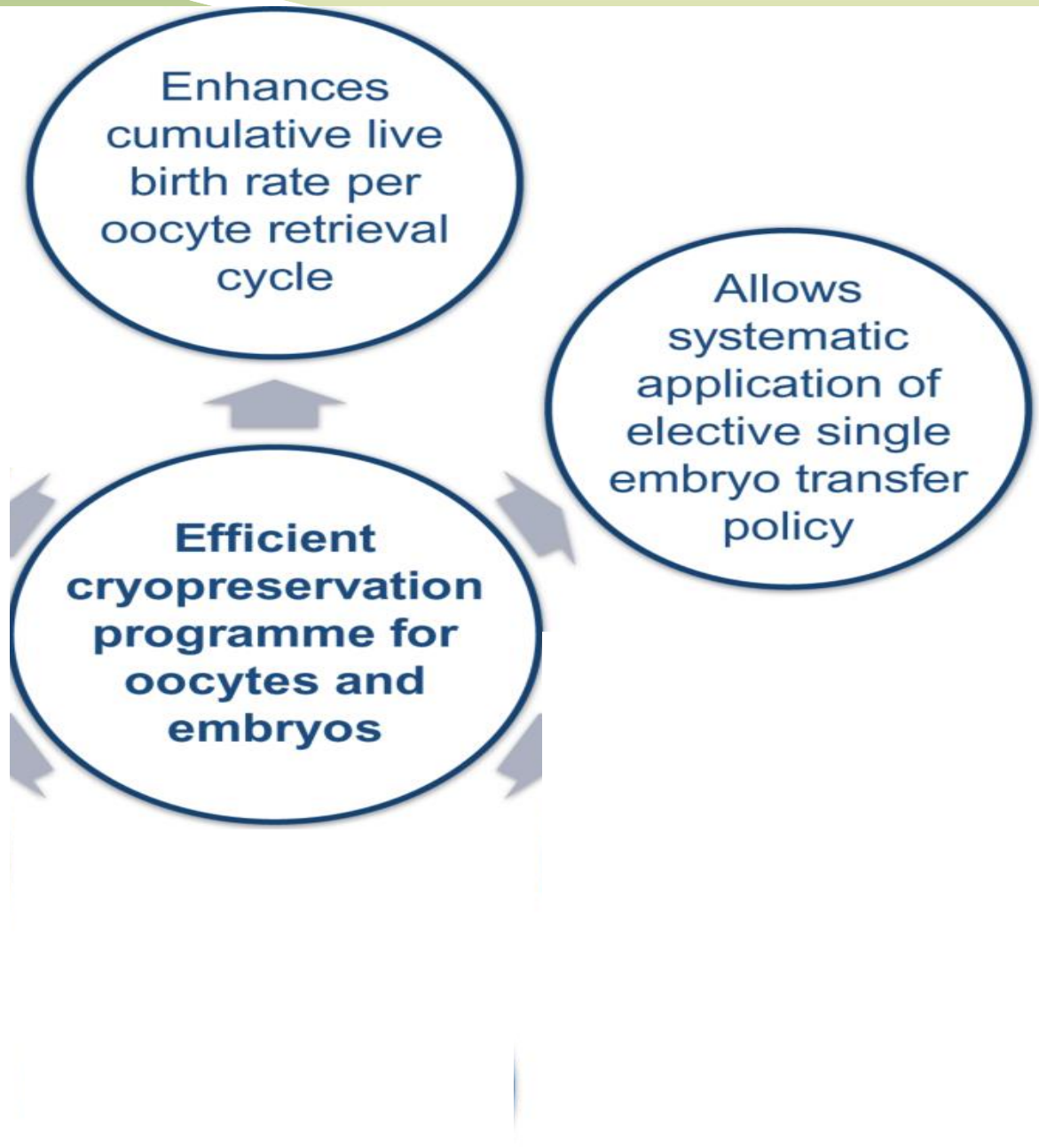
MERCK

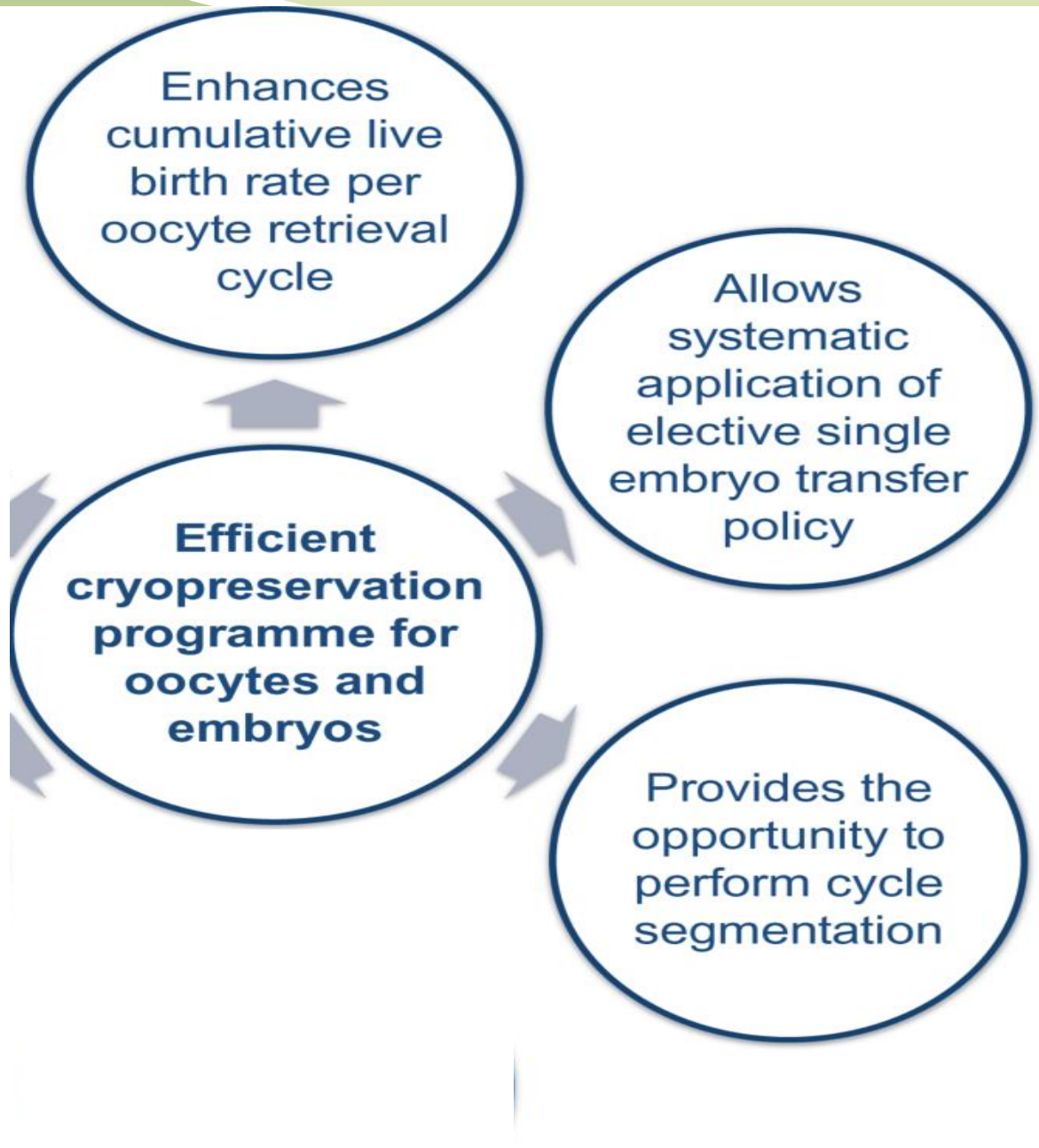
Cryopreservation: the preservation of structurally intact living cells and tissues using very low temperatures

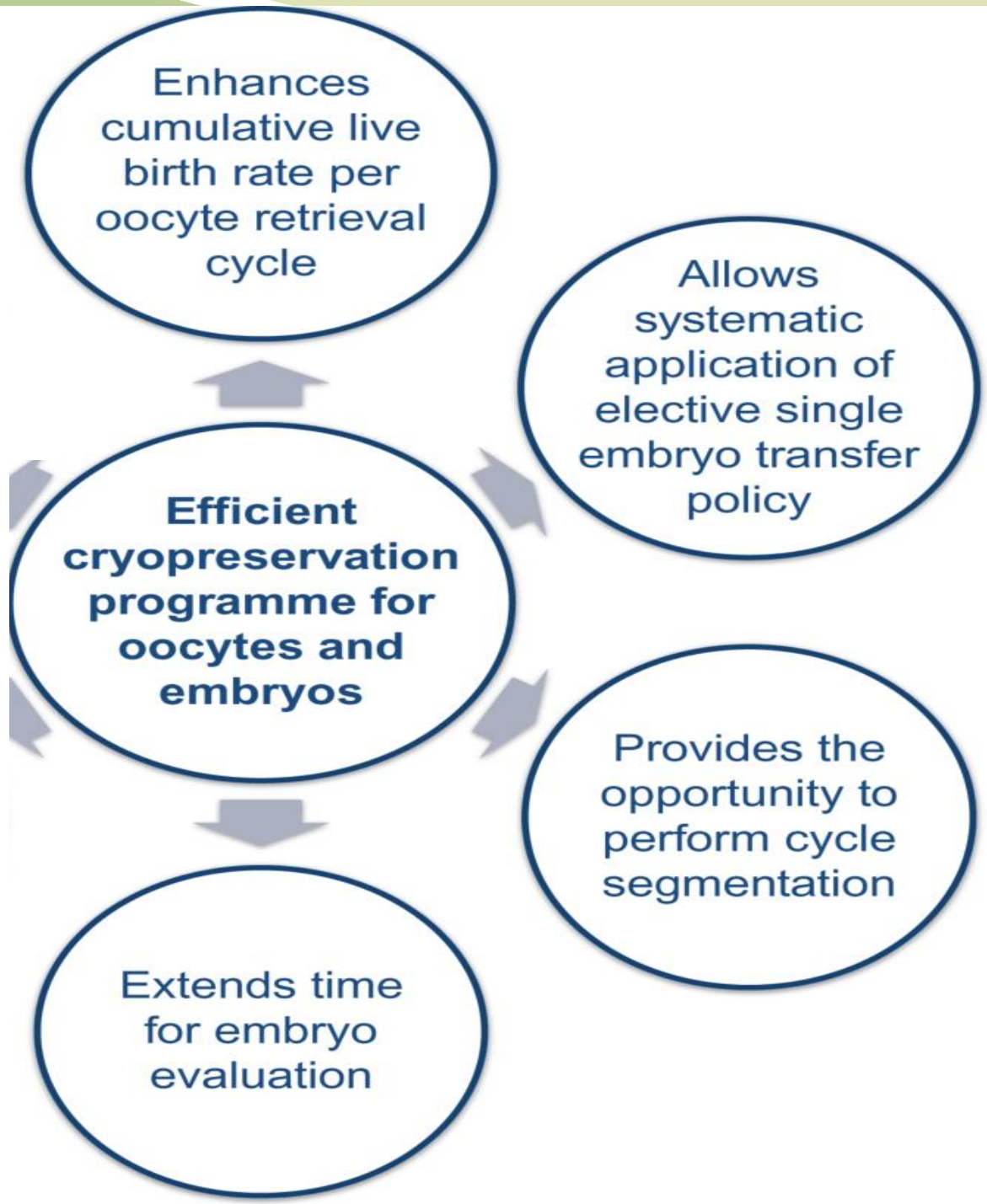
- Sperm and semen - 1953
- Embryos - 1983
  - Cleavage stage
  - Pronucleate stage
  - Blastocysts
- Oocytes – 2000s

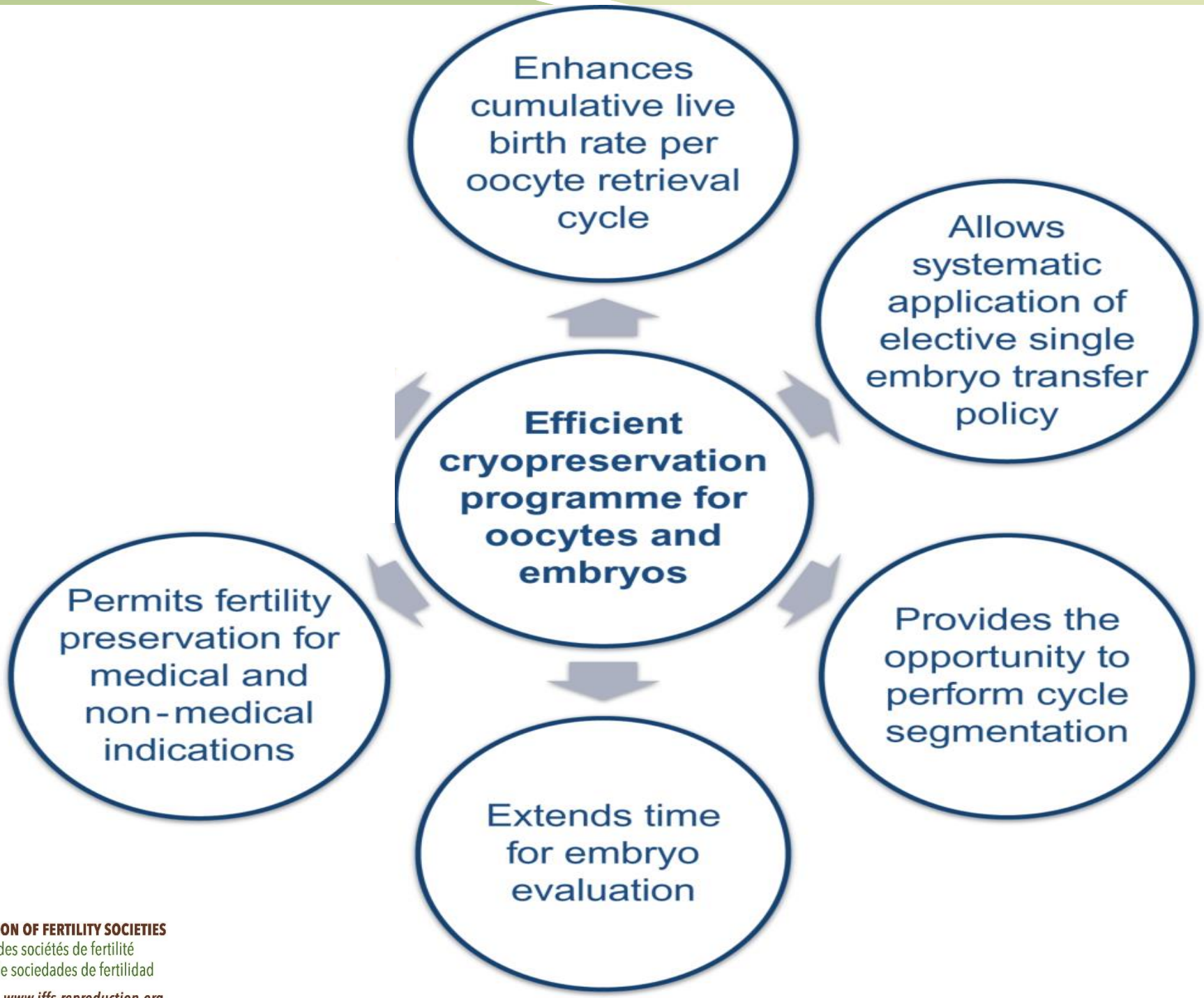

Efficient
cryopreservation
programme for
oocytes and
embryos

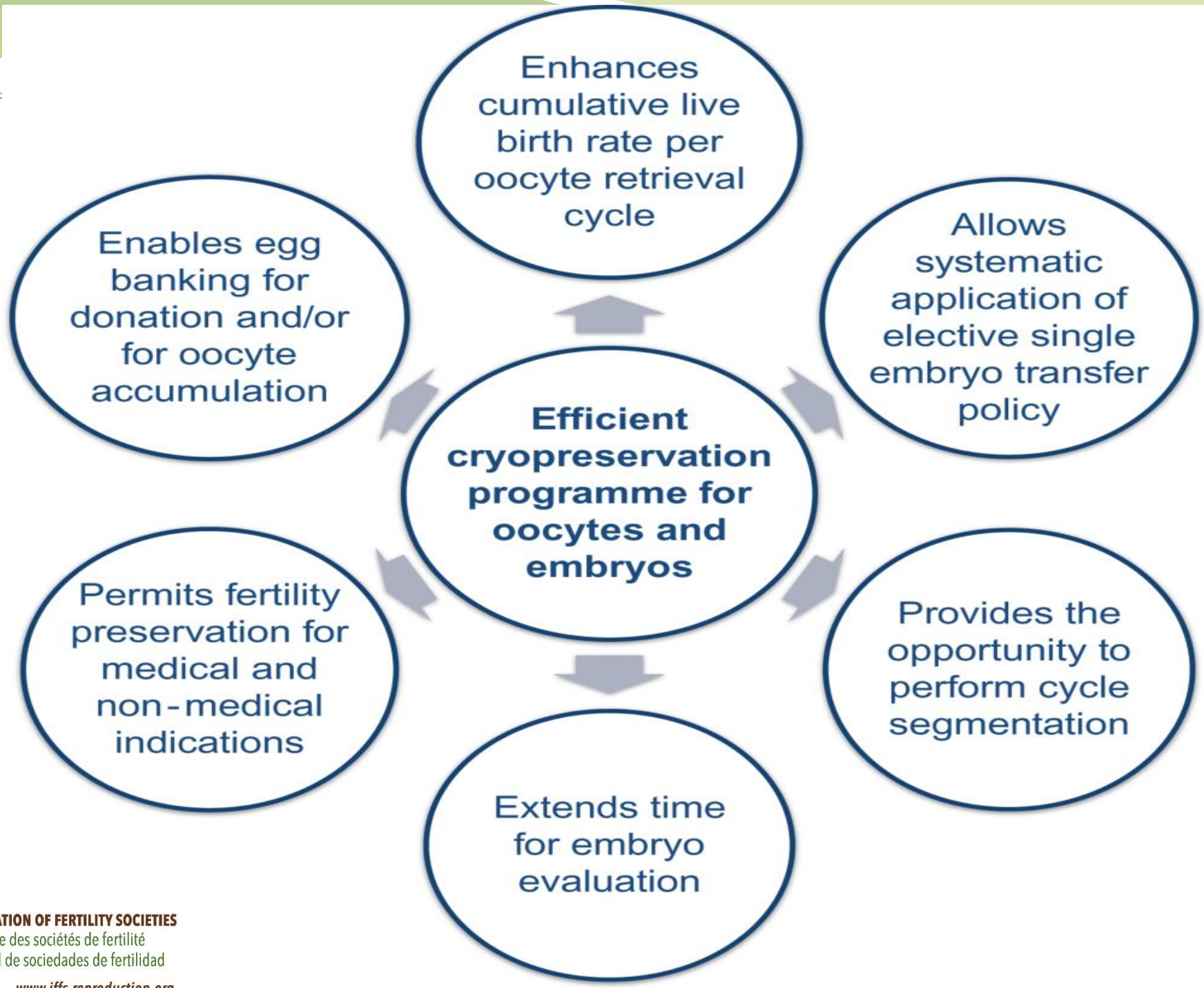

Enhances cumulative live birth rate per oocyte retrieval cycle
Efficient cryopreservation programme for oocytes and embryos


Enhances cumulative live birth rate per oocyte retrieval cycle
Allows systematic application of elective single embryo transfer policy
Efficient cryopreservation programme for oocytes and embryos


Enhances cumulative live birth rate per oocyte retrieval cycle
Allows systematic application of elective single embryo transfer policy
Efficient cryopreservation programme for oocytes and embryos
Provides the opportunity to perform cycle segmentation


Enhances cumulative live birth rate per oocyte retrieval cycle
Allows systematic application of elective single embryo transfer policy
Efficient cryopreservation programme for oocytes and embryos
Provides the opportunity to perform cycle segmentation
Extends time for embryo evaluation


Enhances cumulative live birth rate per oocyte retrieval cycle
Allows systematic application of elective single embryo transfer policy
Efficient cryopreservation programme for oocytes and embryos
Permits fertility preservation for medical and non-medical indications
Provides the opportunity to perform cycle segmentation
Extends time for embryo evaluation


Enhances cumulative live birth rate per oocyte retrieval cycle
Allows systematic application of elective single embryo transfer policy
Provides the opportunity to perform cycle segmentation
Extends time for embryo evaluation
Permits fertility preservation for medical and non-medical indications
Enables egg banking for donation and/or for oocyte accumulation
Efficient cryopreservation programme for oocytes and embryos

Methods for Embryo Cryopreservation

Slow Freezing

Vitrification

Slow Freezing and Vitrification: The similarities

- Preserve cells at low temperature (-196°C)
- Prevent ice crystal formation - dehydration
- Use cryoprotectants
  - Non-permeating – dehydration by osmosis
    - Sucrose
    - Trehalose
  - Permeating – replace water inside the cells
    - Propanediol (PROH)
    - Glycerol
    - DMSO

Slow freezing

- Uses programmed freeze machine
- Gradual reduction in temperature
- Gradual dehydration
- Up to 20 embryos simultaneously
- Consistent for all 20 embryos
- Each freeze run takes ~1.5hrs

Vitrification

- No machine required
- Rapid reduction in temperature
- Solidification of cells to form glass-like state
- Vitrify individually (label individually)
- Technically challenging
- Inter-procedure (inter-embryo) variability
- ~15mins per embryo

To Slow Freeze *or* To Vitrify Embryos?

Comparison of Slow Freezing and Vitrification

Risks and costs

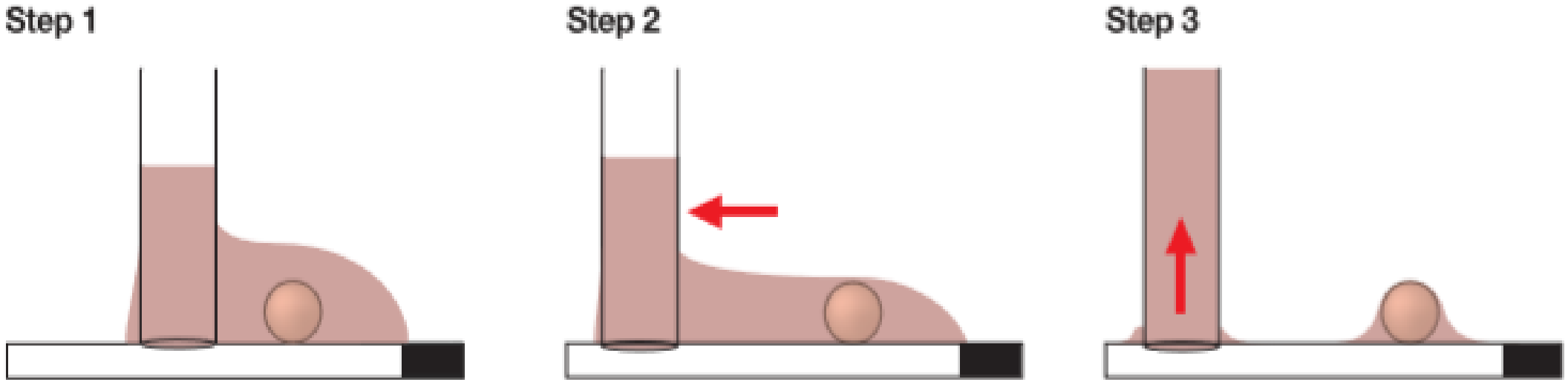
Risk	Slow Freezing	Vitrification
Cryoprotectant toxicity	+	+++
Osmotic stress	++	+++
Solution effect toxicity	+++	-
Intracellular ice formation	+++	-
Accidental thawing risk	-	++
Technology dependent	+++	-
Practitioner dependent	+	+++
Consumables cost	+	+++
Storage space	+	+++

Time costs	Slow Freezing	Vitrification
Dehydration	10-15 minutes	10-15 minutes
Straw loading	<2 minutes	<1 minute
Seeding	1 minute	-
Cooling to -196°C	1-2 hours	Instant
Place in storage tank	5 minutes	5 minutes
Warming	30 minutes	10-15 minutes
Multiple embryos simultaneously	YES	NO

Vitrification devices

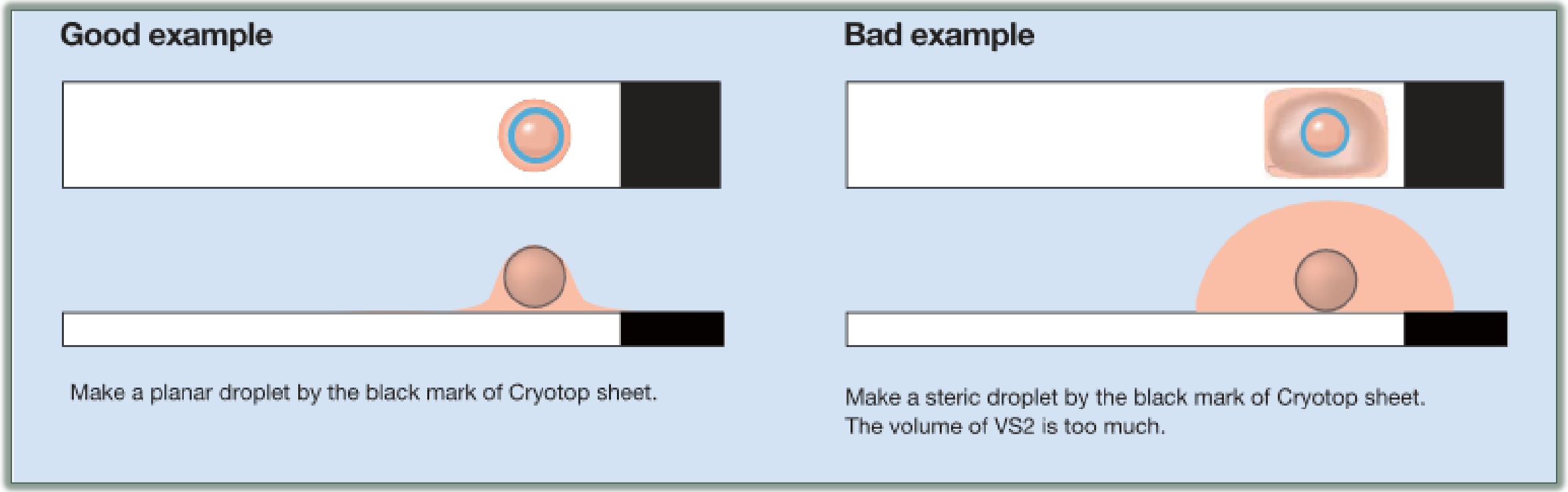
Vitrification – technical challenges

Placing the embryo on the holding device



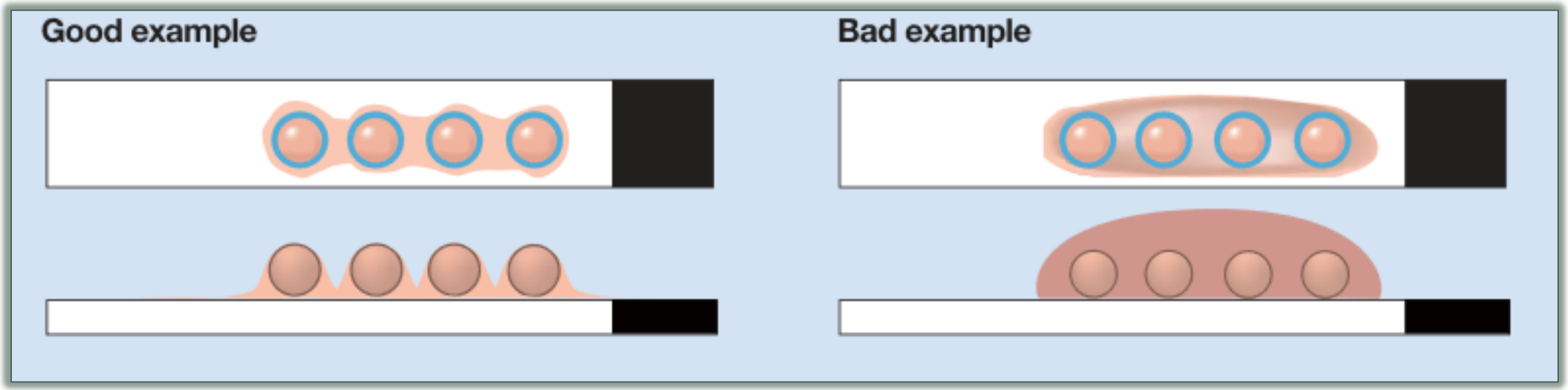
Vitrification – technical challenges

Removal of excess vitrification solution



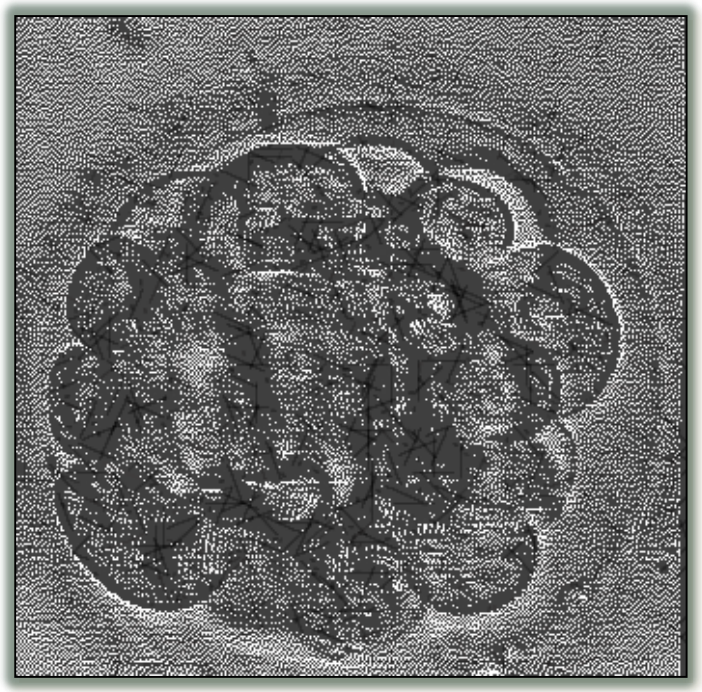
Vitrification – Technical challenges

Removal of excess vitrification solution

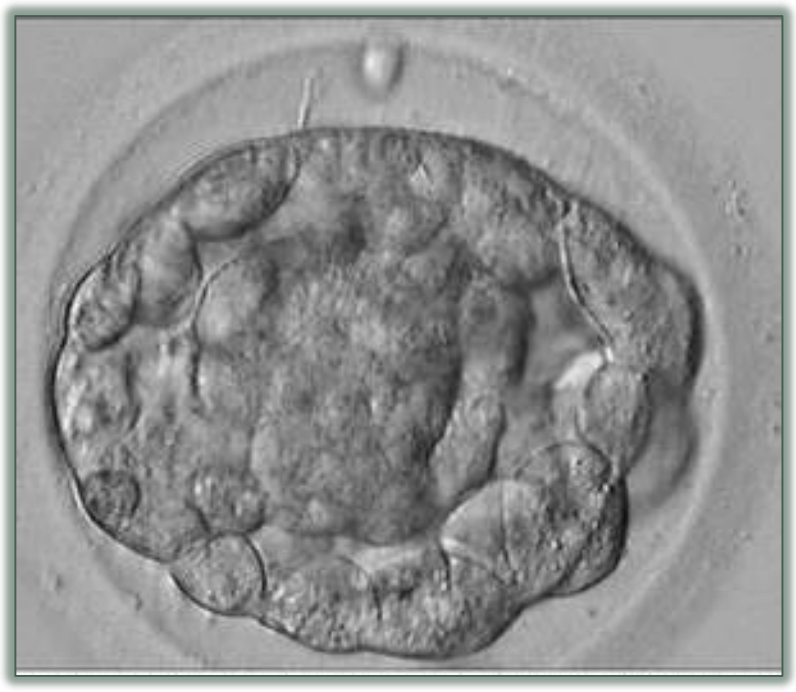


To Slow Freeze *or* To Vitrify? Assessment of embryo survival

“Survival” incudes Re-expansion



Time zero



After up to 2 hours

Human Reproduction Update, Vol.23, No.2 pp. 139–155, 2017
Advanced Access publication on November 4, 2016 doi:10.1093/humupd/dmw038

human reproduction update

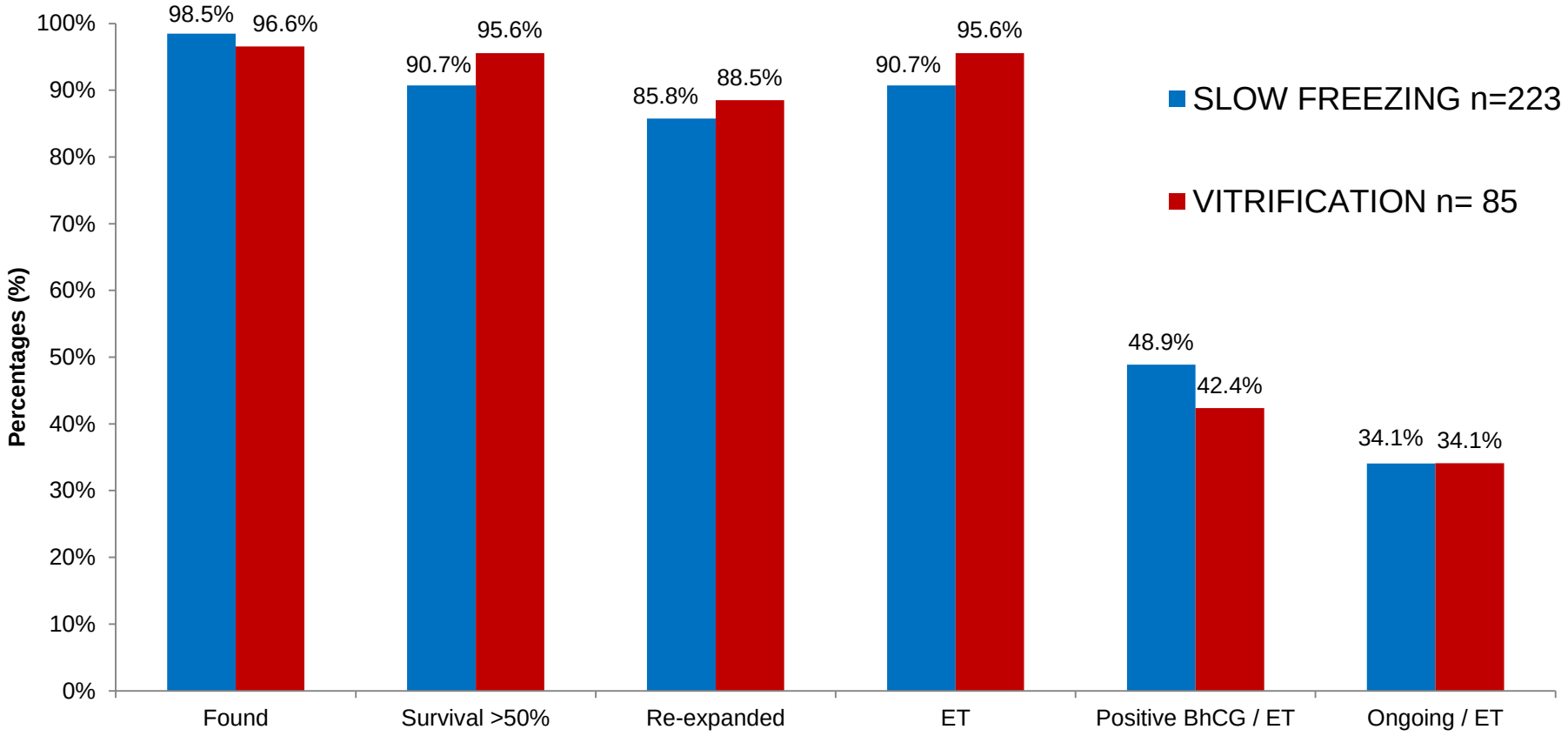
Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance

Laura Rienzi[1,*], Clarisa Gracia[2], Roberta Maggiulli[1], Andrew R. LaBarbera[3], Daniel J. Kaser[4], Filippo M. Ubaldi[1], Sheryl Vanderpoel[5,6], and Catherine Racowsky[4]

WIDER IMPLICATIONS: Data from available RCTs suggest that vitrification/warming is superior to slow-freezing/thawing with regard to clinical outcomes (low quality of the evidence) and cryosurvival rates (moderate quality of the evidence) for oocytes, cleavage-stage embryos and blastocysts. The results were confirmed by cohort studies. The improvements obtained with the introduction of vitrification have several important clinical implications in ART. Based on this evidence, in particular regarding cryosurvival rates, laboratories that continue to use slow-freezing should consider transitioning to the use of vitrification for cryopreservation.

Outcomes of Slow Freezing & Vitrification

Blastocysts Cryopreserved Jan-June 2017



To Slow Freeze or To Vitrify Embryos?

To Slow Freeze *or* To Vitrify Embryos?

- Clinical outcomes are similar
- Each method has advantages and disadvantages
- **Method of choice must be based on abilities and facilities available to provide the best service for patients**
- Assessment of embryo survival is an *intermediate* measure
- The true measure of success is the birth of a healthy child

www.iffs-reproduction.org @IntFertilitySoc nt@FedFertilitySoc