Vitrification of reproductive cells: The next breakthrough in ART?

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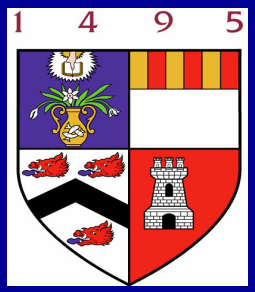

"breakthrough"

"Some signal achievement in scientific research"

but beware

"……the word is being overused"

(Fowler's English Usage)

"A sudden important development or success"

(Oxford Dictionary)

"A major achievement or success that permits further progress, as in technology."

(American Heritage Dictionary of English)

Question

- Is vitrification a *“breakthrough”?*
- Does it progress ART further than equilibrium rate freezing has done?

Claims made for vitrification

Quicker

Simpler

More efficient

Less expensive

More successful

than equilibrium rate freezing

Do we have the evidence to support these claims?

How successful is vitrification in practice?

- Practical advantages?
- Robust?

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- Practical advantages?
- Robust?
  - Demands on embryologist
  - Efficiency
  - Cost
  - Safety of recipients/storage/transport

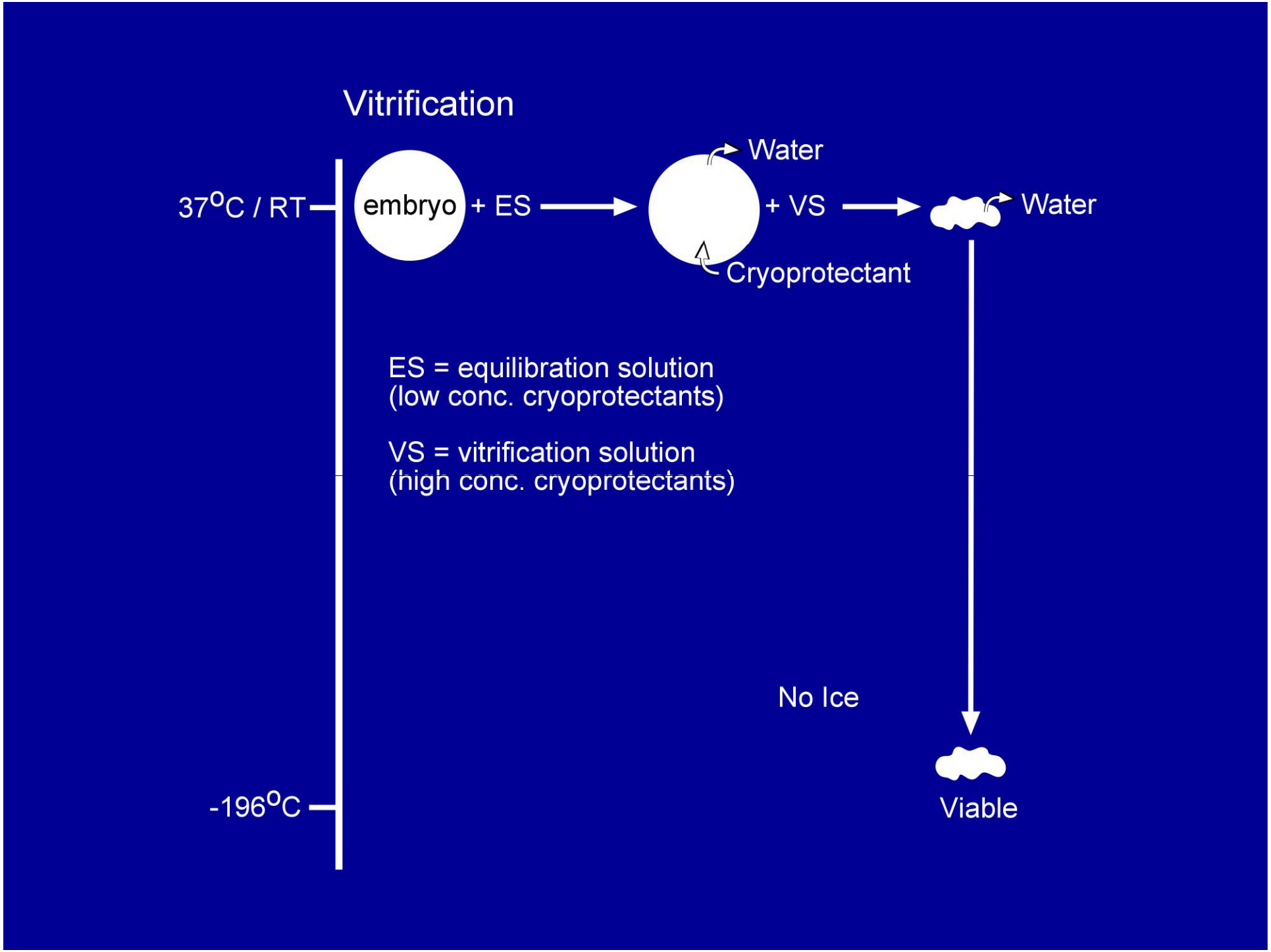
How successful is vitrification in practice?

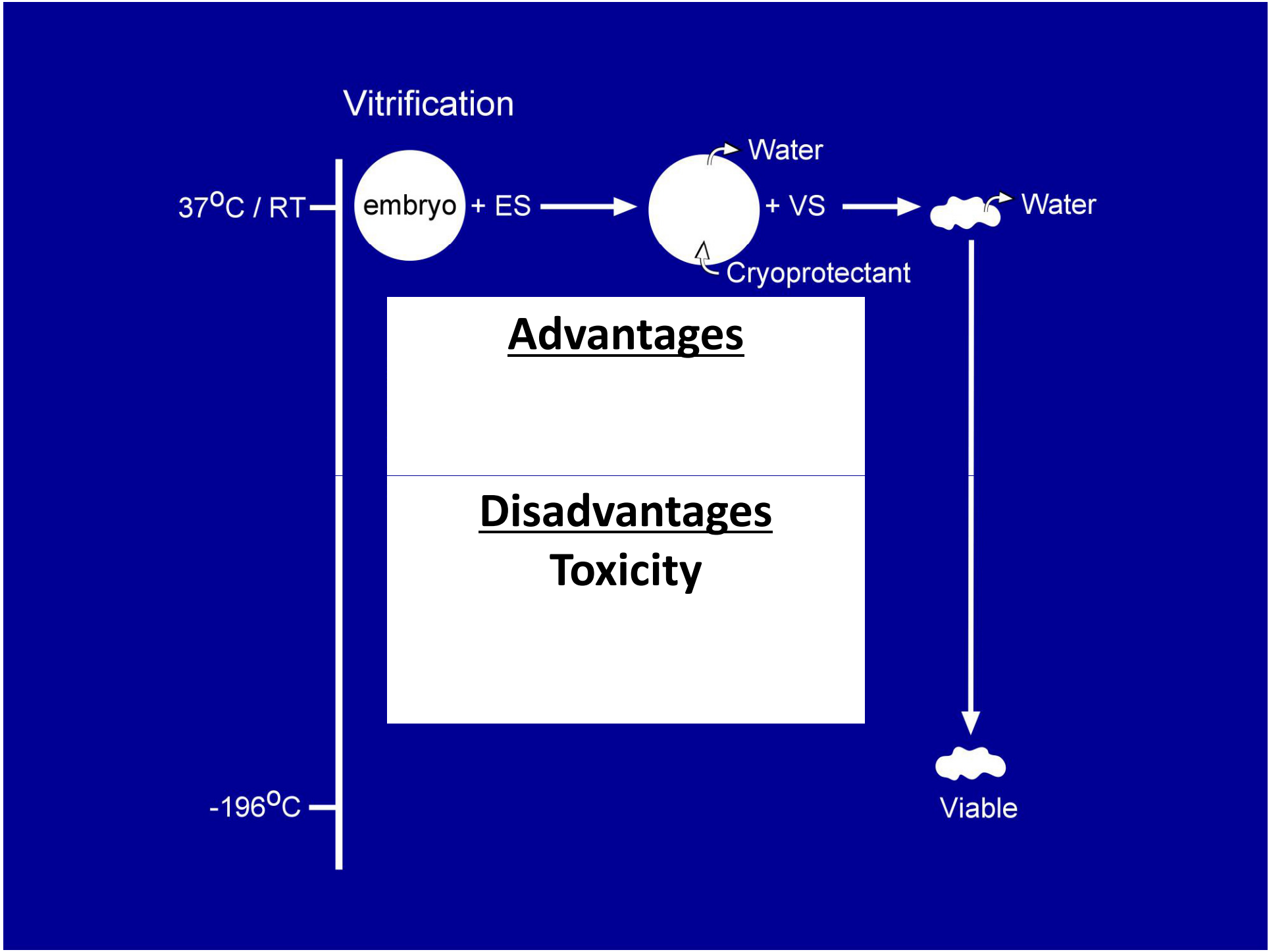
- Practical advantages?
- Robust?
- Improved survival?
- Improved live birth rate?
- Safe for children conceived?

Reproductive cells vitrified

- Testicular tissue?
- Ovarian tissue
- Sperm
- Oocytes (GV and MII)
- Embryos

Vitrification in the laboratory


Vitrification
37°C / RT
embryo
+ ES
Water
+ VS
Cryoprotectant
Water
ES = equilibration solution
(low conc. cryoprotectants)
VS = vitrification solution
(high conc. cryoprotectants)
No Ice
Viable
-196°C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Advantages
Disadvantages
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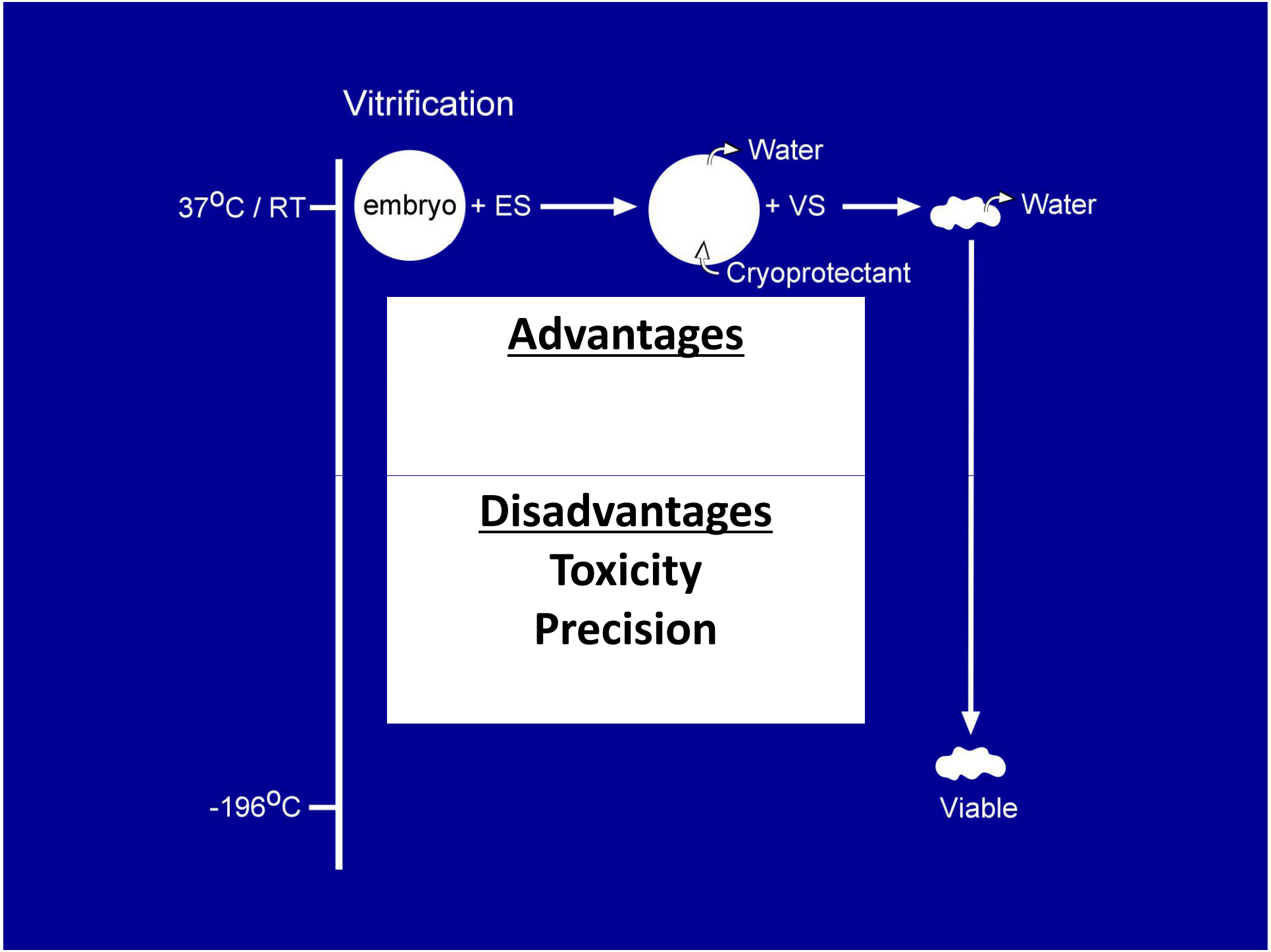
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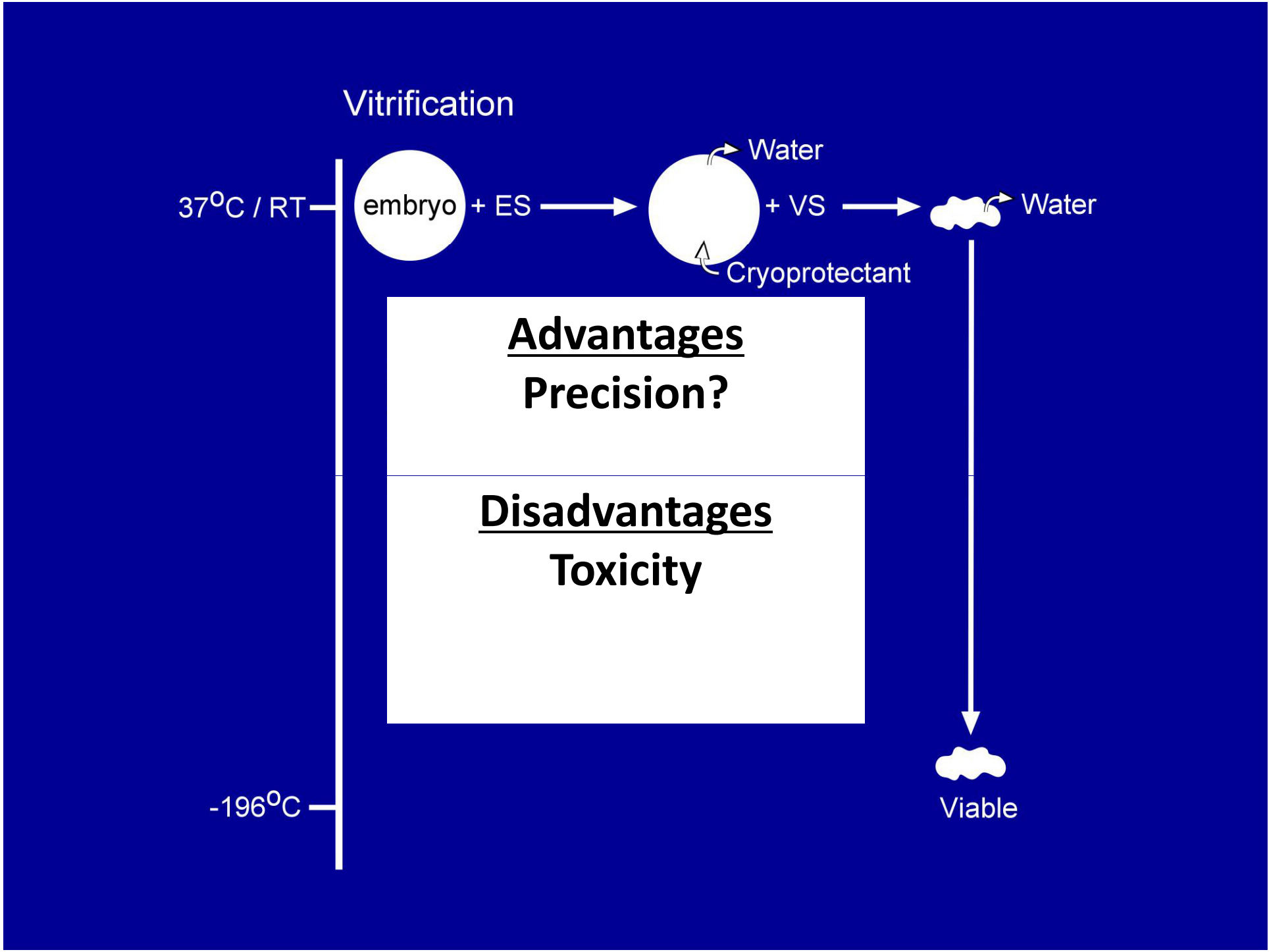
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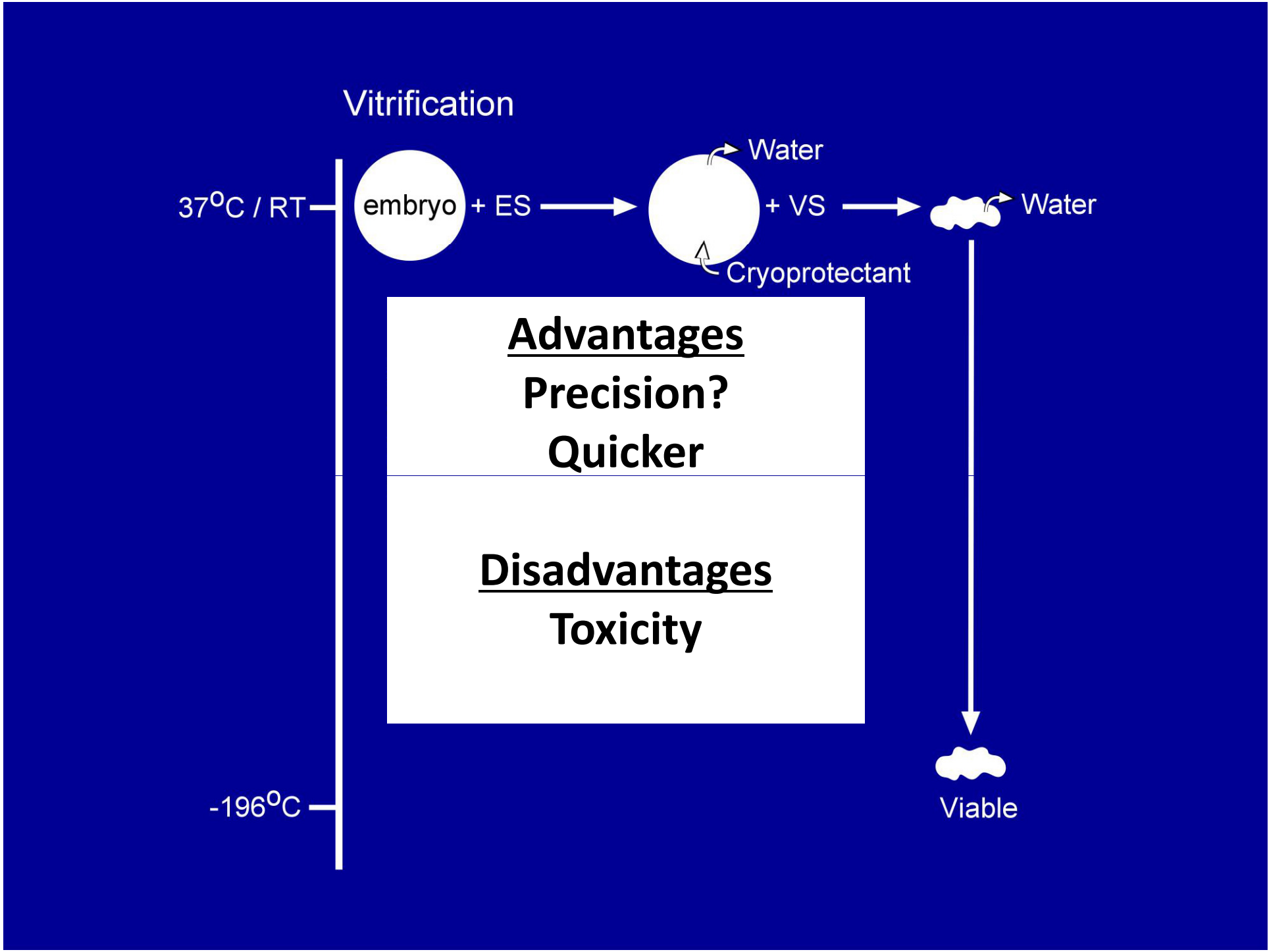
Requirements in the Lab

- Training
- Regular practice
- Uninterrupted concentration
  - –Dedicated quiet area?
  - –Additional staff?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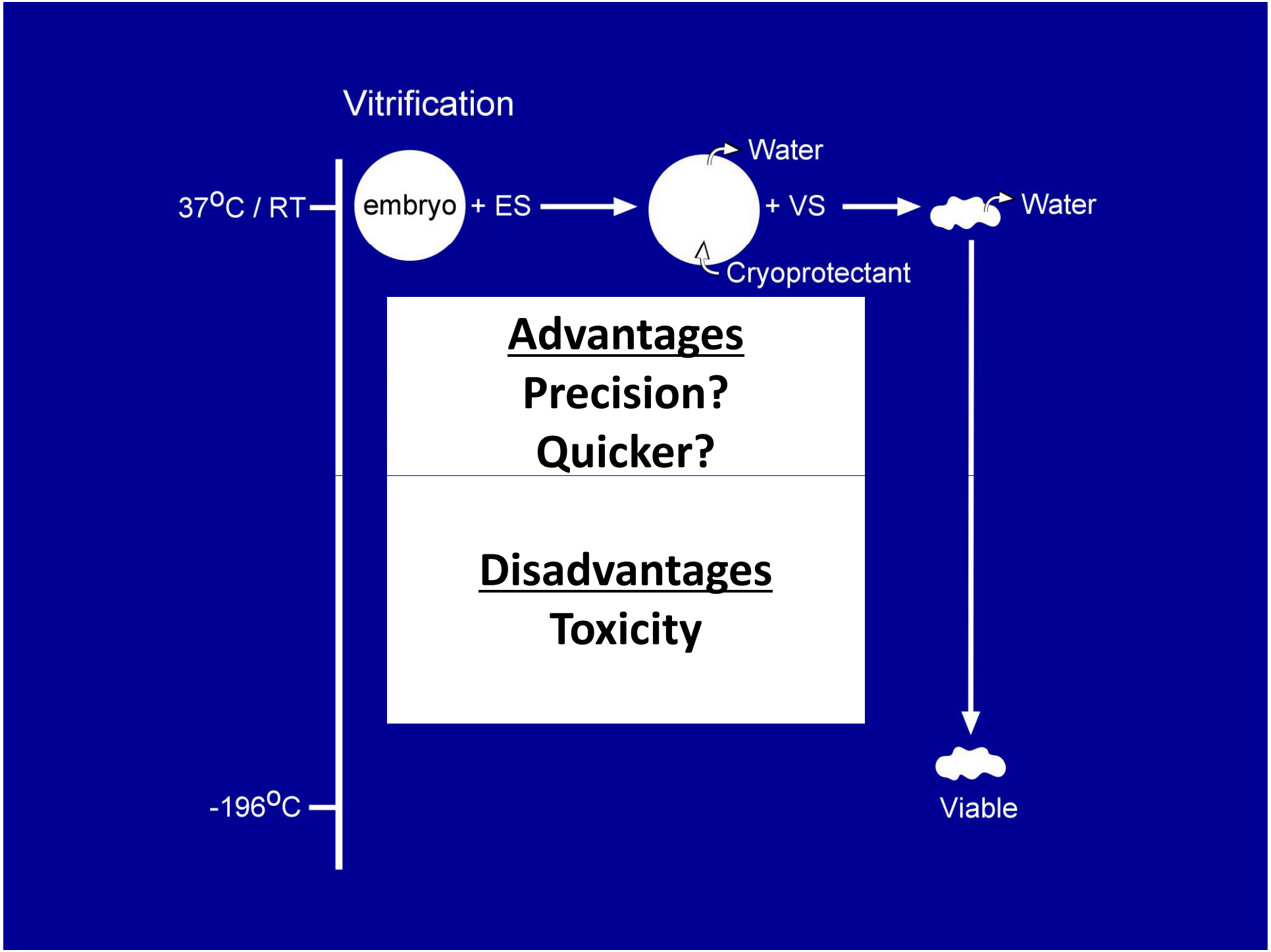
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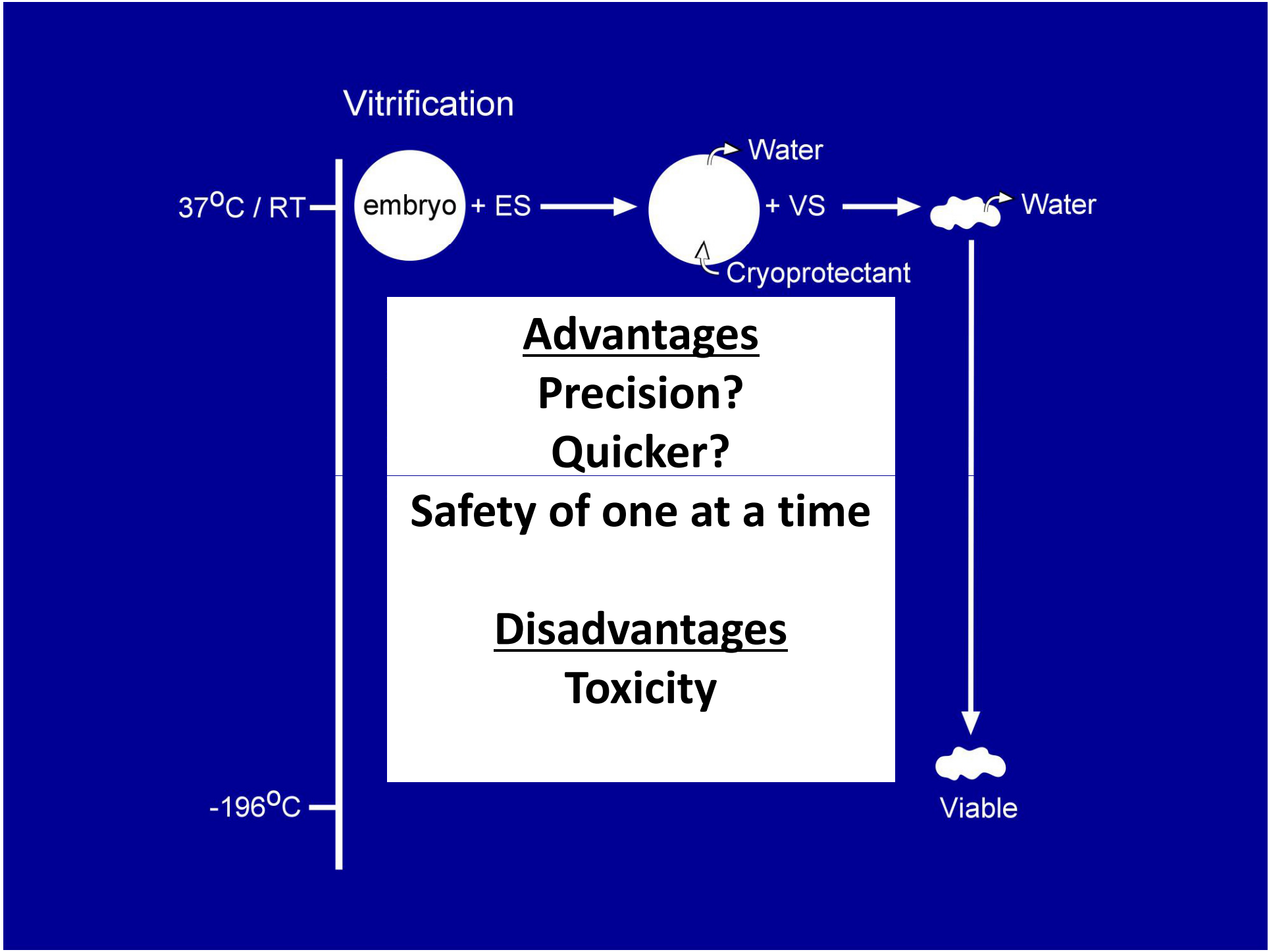
Increased efficiency for large groups?

- Load >1-2 oocytes/embryos
  - –but hi survival and increased eSET
  - –waste surplus
- Equilibrate large groups
  - –validated?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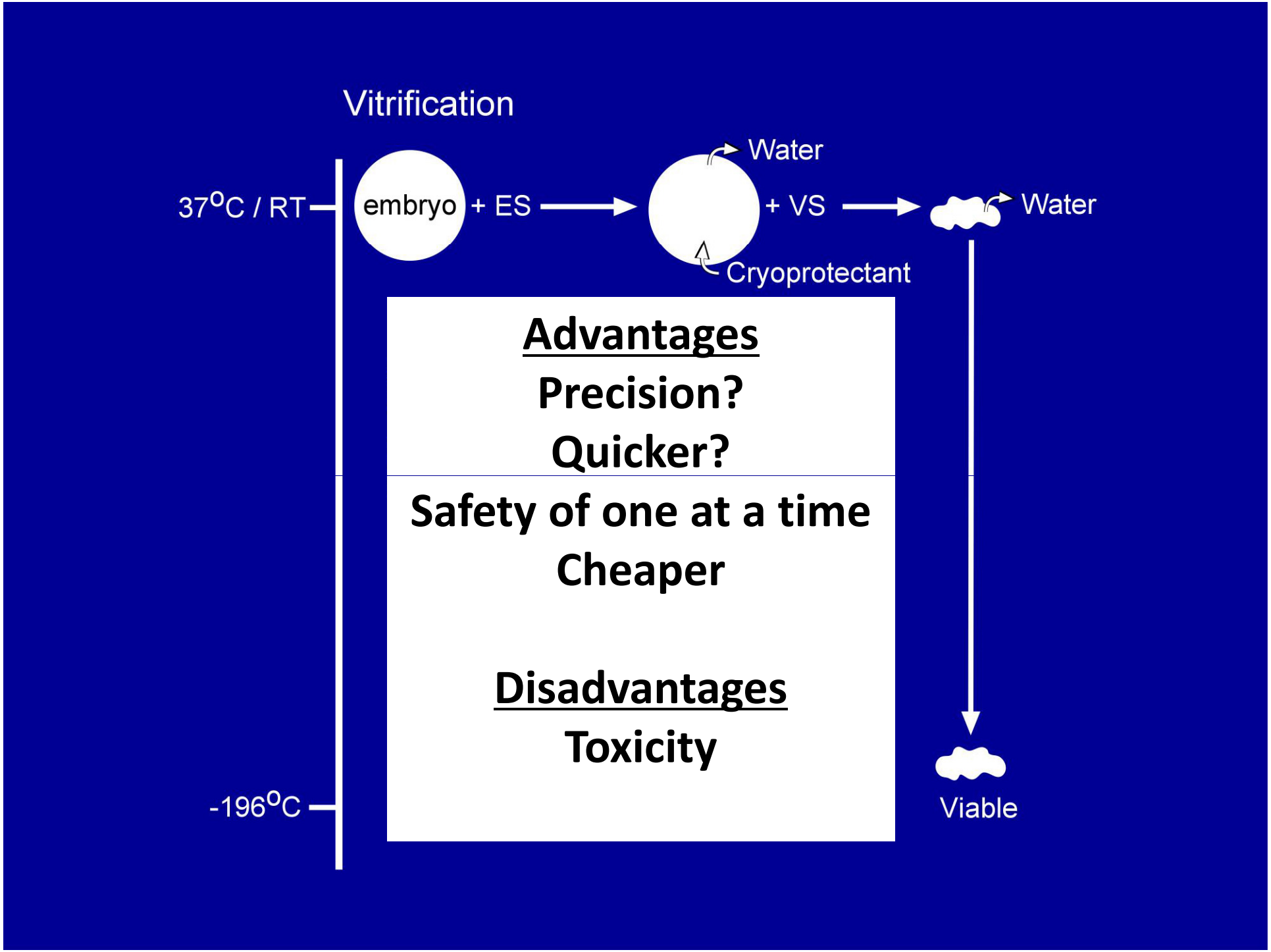
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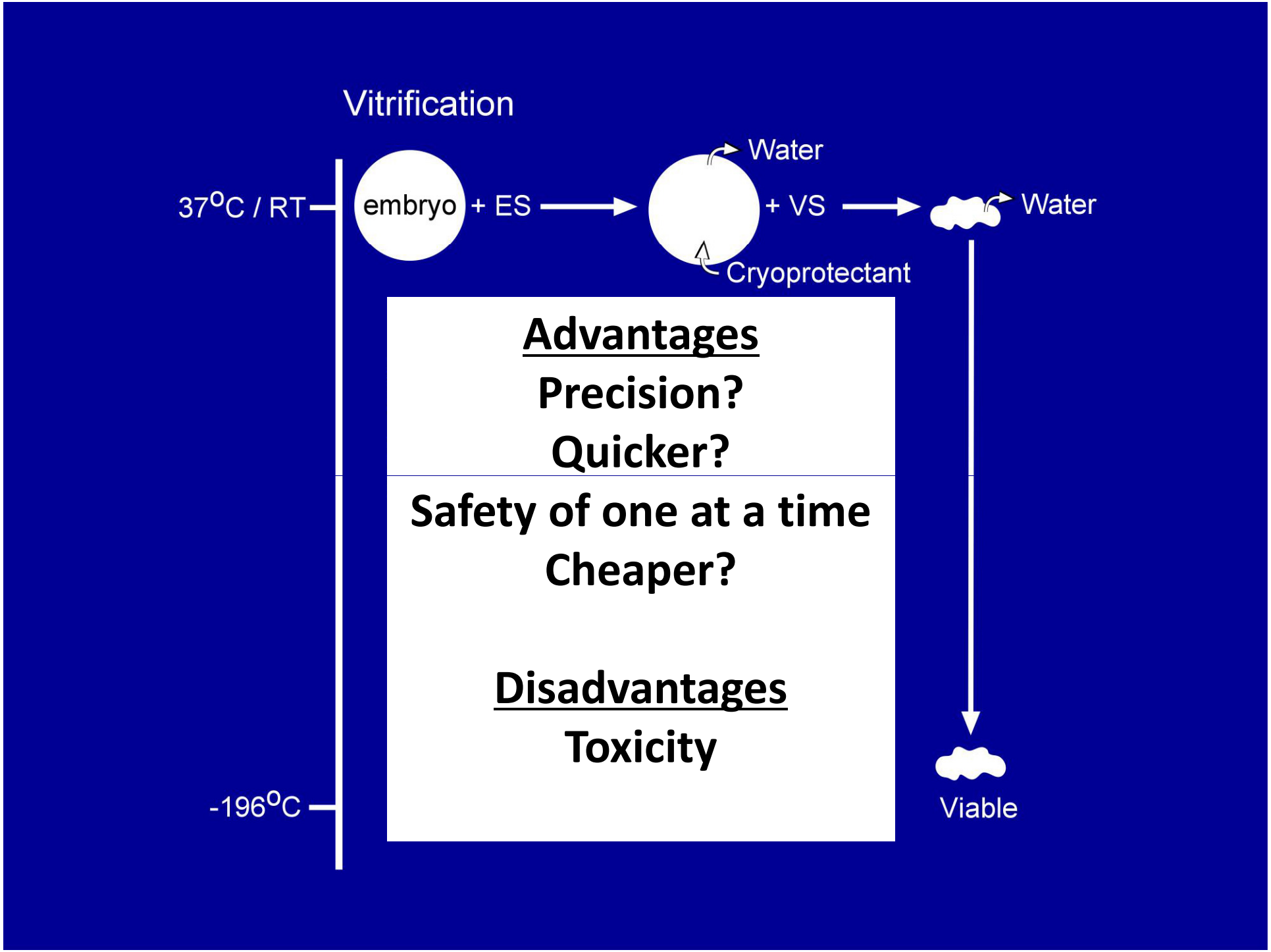
Is vitrification cheaper?

- No freezing machine
- Costly consumables
- More embryologists' time
- Capital outlay *vs* running costs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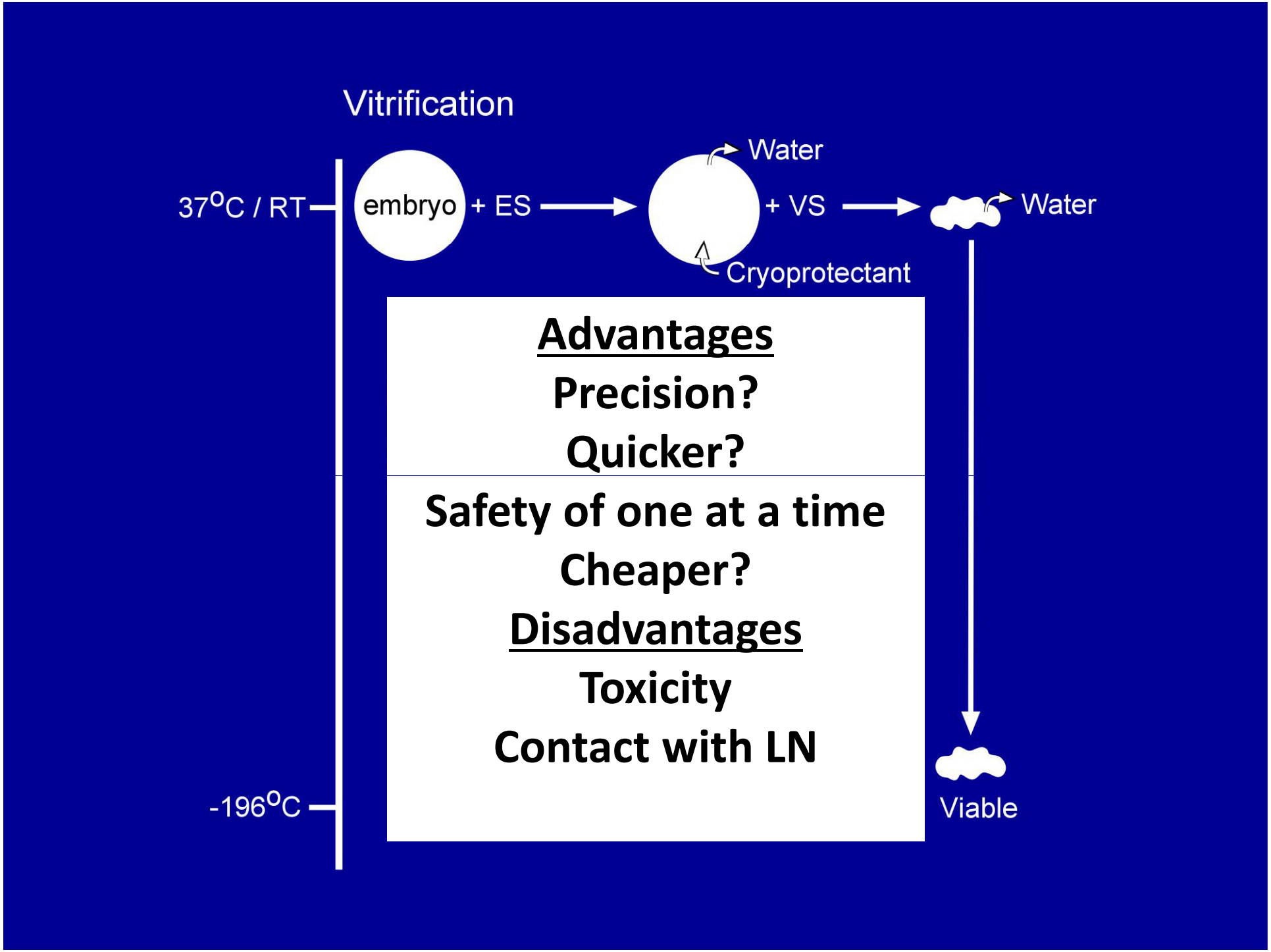
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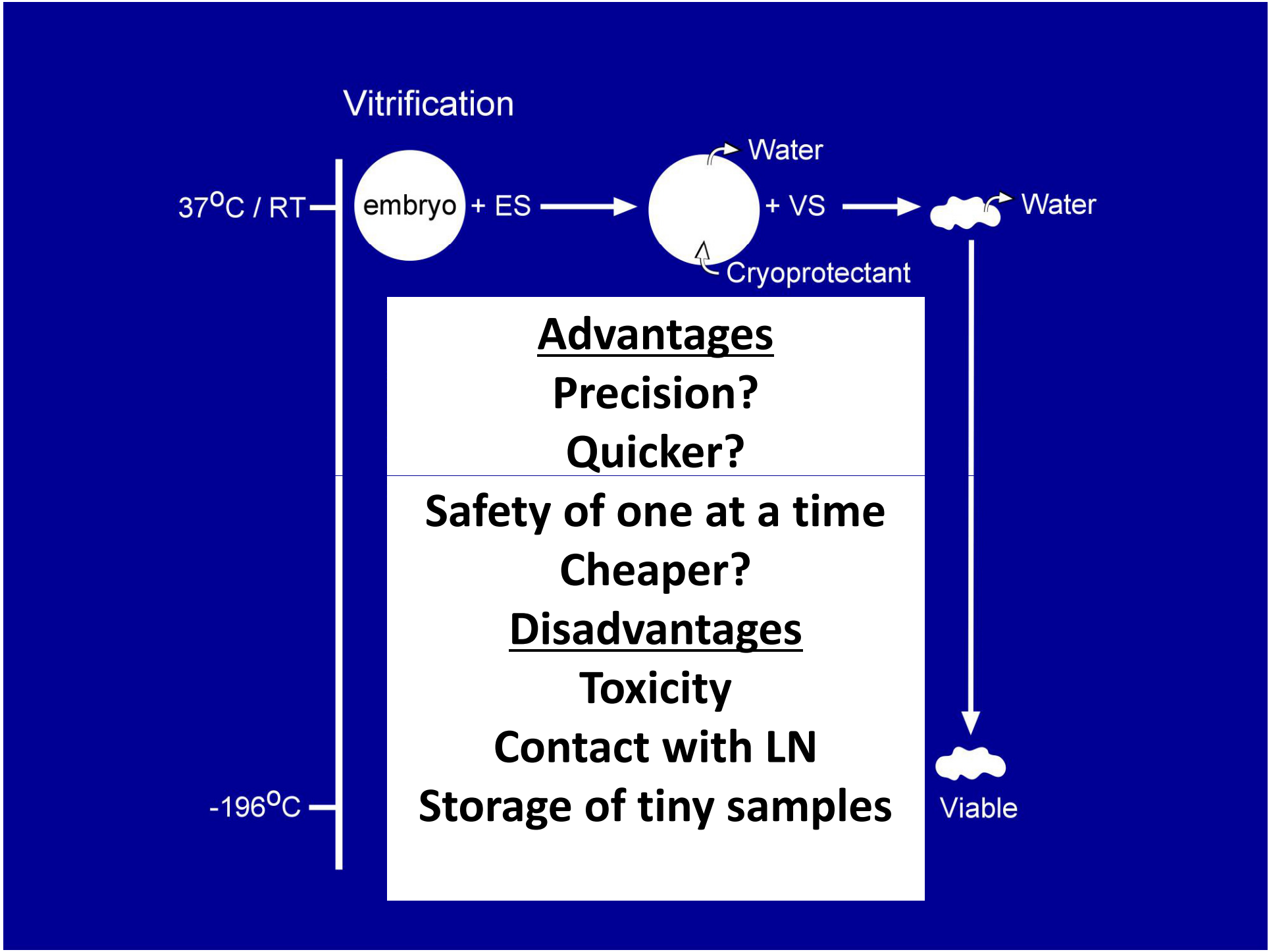
Open *vs* closed containers

- Do closed containers ↓survival?
- Confront the problem
- More research
- Do not compromise practice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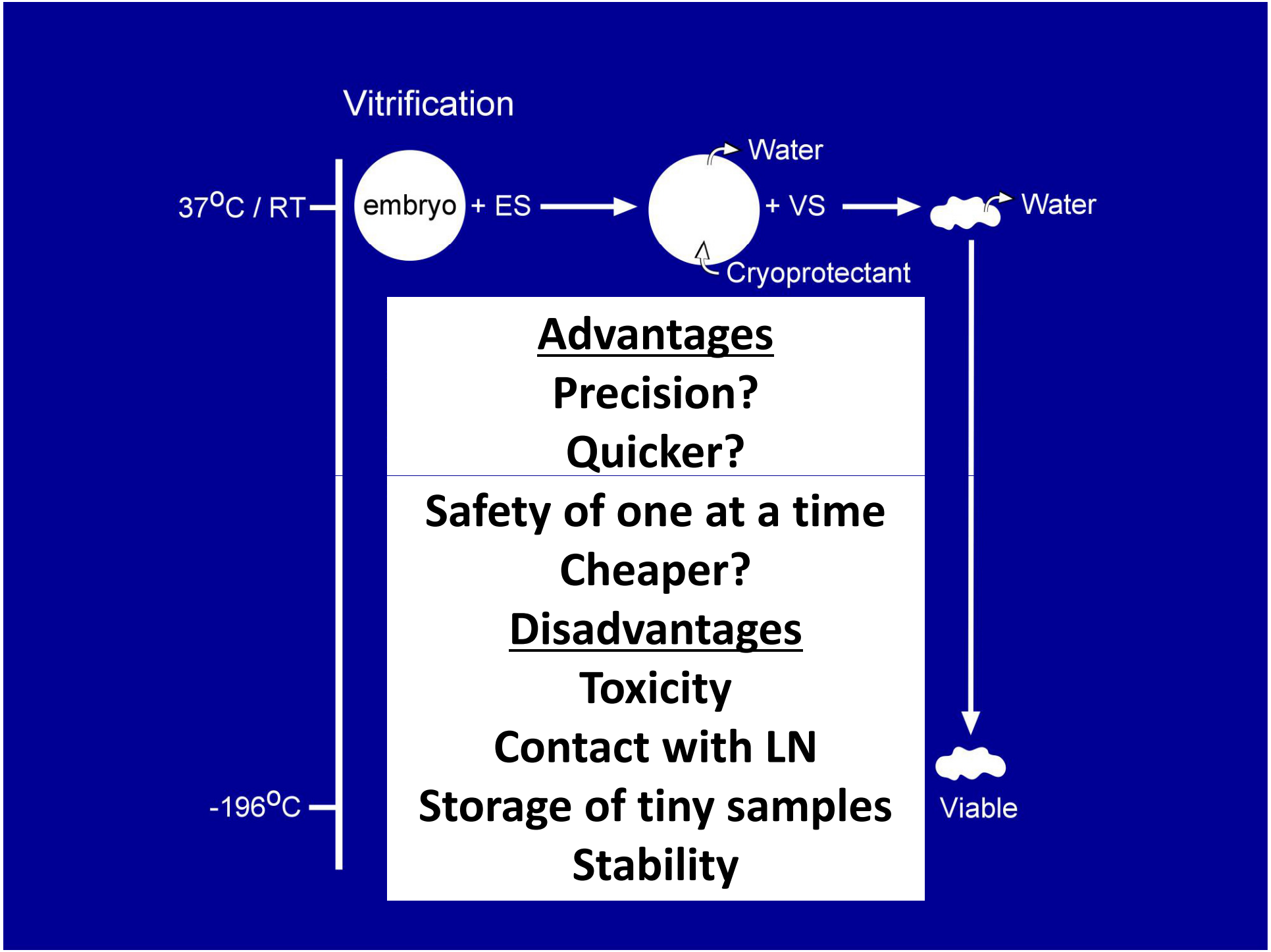
Storage of tiny samples
-196°C
Viable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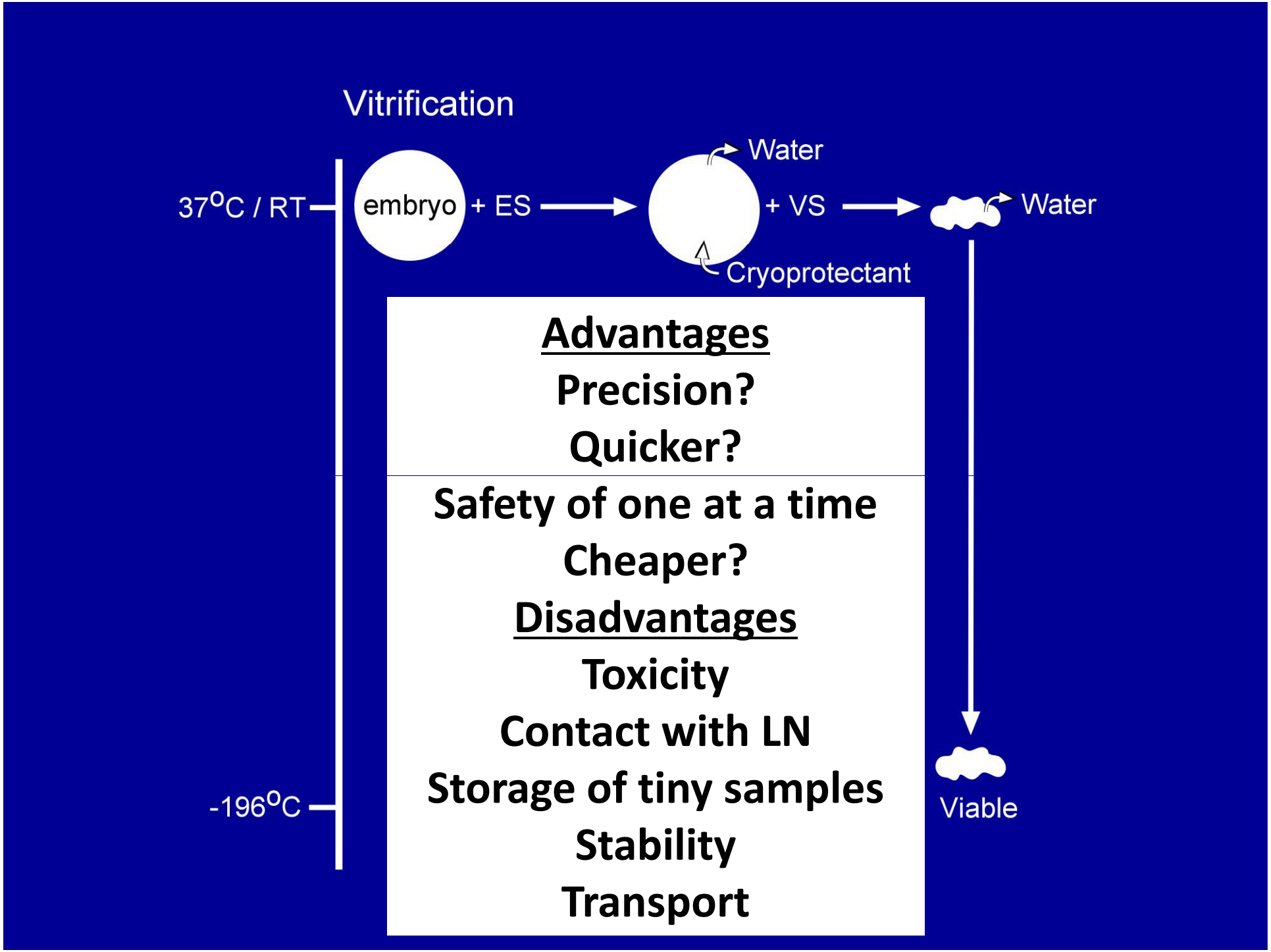
Storage of tiny samples
Stability
-196°C
Viable

Storage of vitrified samples

- Frozen gametes and embryos
  - –viable after many years
- Vitrified oocytes and embryos
  - –long term safety not proven
  - –follow up essential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Transport
-196°C
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Transport

- Warn patients of risks
- Preparation of shipper
- Strict control during transport
- Speed moving samples in and out
- Precise protocols in advance
- Practise

Vitrification in clinical practice

How successful is vitrification in practice?

- Practical advantages?
- Robust?
- **Improved survival?**
- **Improved live birth rate?**
- Safe for children conceived

Post-warming survival of cryopreserved embryos

	Vitrification (N)	Freezing (N)	Odds Ratio [95% CI]
Cleavage stage*	94% (490)	74% (484)	6.35 [1.14-35.26]
Blastocyst Stage**	90% (252)	70% (217)	4.09 [2.45-6.84]

* 4 RCT; ** 2 RCT

From Kolibianakis *et al* (2009) Current Opinions in Obstet and Gynaecol. 21:270

Blastocyst development from cryopreserved embryos*

Vitrification (N)	Freezing (N)	Odds Ratio [95% CI]
53% (268)	48% (214)	1.56 [1.07-2.27]

* 2 RCT

Meta-analysis by Kolibianakis *et al*, 2009

Clinical Outcome - Embryos

Clinical pregnancy: 3 RCT

OR 1.66, 95% CI 0.98-2.79

(Kolibianakis *et al*, 2009)

Live birth: 1 RCT

33% (N=40) *vs* 13% (N=23)

(Rama Raju *et al*, 2005)

Survival of biopsied embryos

Method	Embryos (N)	% Survived	% Blastocyst
Slow freeze	Control (53)	85	20
Slow freeze	Biopsied (52)	16	2
Modified freeze	Biopsied (52)	75	23
Modified thaw	Biopsied (50)	76	14
Vitrification	Biopsied (49)	94	18

Zheng *et al* 2005

Timely to revisit some of our freezing protocols?

“More than one way to skin a cat”

Oocyte vitrification

- Theoretically vitrification offers better protection than freezing

Oocyte vitrification

- Theoretically vitrification offers better protection than freezing
- Results encouraging:
  - Hi rates survival
  - >392 children born
- 1 completed RCT and 1 ongoing
- Reserve judgement

Is vitrification of reproductive cells riskier than freezing for the children conceived?

We do not know!

Cryopreservation follow-up

		Number of births	Follow-up
Embryos	Frozen	Many thousands	↑ Major malformation?
	Vitrified	Several thousand	?
Oocytes	Frozen	>532	6 birth defects
	Vitrified	>392	6 birth defects

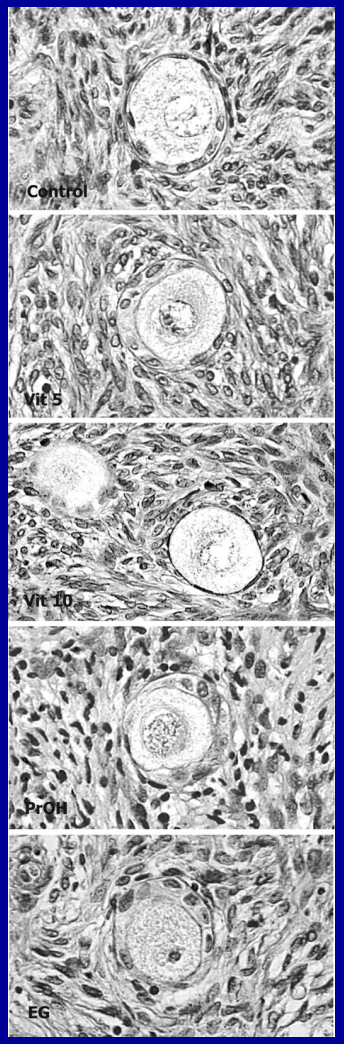
Risks of cryopreservation

- Possibility epigenetic effects?
- Long term follow-up
- Open mind

Vitrified ovarian tissue

- **Preserve architecture and cell connections >**

Light microscopic images of non-frozen (Control), vitrified (Vit 5 and Vit 10) and cryopreserved human ovarian cortex after using slow freezing programmes with PrOH and EG cryoprotectants



Control

Vitrified

Frozen

Keros, V. et al. Hum. Reprod. 2009 24:1670-1683; doi:10.1093/humrep/dep079

Human Reproduction

Vitrified ovarian tissue

- Preserve architecture and cell connections
- Freezing and vitrification → similar preservation oocytes and granulosa cells
- Vitrification → better preservation stroma
- No extensive functional tests
- No clinical use

Sperm vitrification

- Open/closed container
- No penetrating CPA
- Moderate – ultra-rapid cooling
- Rapid warming
- Fertilisation → blastocyst
- No clinical application

Is vitrification better than freezing?

	Embryo	Oocyte	Sperm	Ovarian tissue
Laboratory	Yes/No	Yes/No	?	Probably
Storage				
Survival				
Live birth				
Safety for children				

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Survival	Yes?	Possibly	Similar	Possibly
Live birth				
Safety for children				

Is vitrification better than freezing?

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Laboratory	Yes/No	Yes/No	?	Probably
Storage	?	?	?	?
Survival	Yes?	Possibly	Similar	Possibly
Live birth	?	?	ND	ND
Safety for children	?	?	ND	ND

Summary

- Is vitrification better than freezing?
  - Lack of sound evidence
  - Prospective randomised comparisons
  - Focus on live birth rates
  - Assess “robustness” in various clinics
  - Safety during storage
  - Re-examine freezing protocols

Conclusion

Vitrification “*very promising*”

but not yet

a “*breakthrough*” in ART