Io or Ganymede:

"Are we going to see major changes in the Orbit of Vitrification"

Southwest Embryology Summit

January 9-11, 2025

Sheraton Mesa at Wrigleyville West, Mesa, AZ

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for Reproductive Health | Chicago, USA

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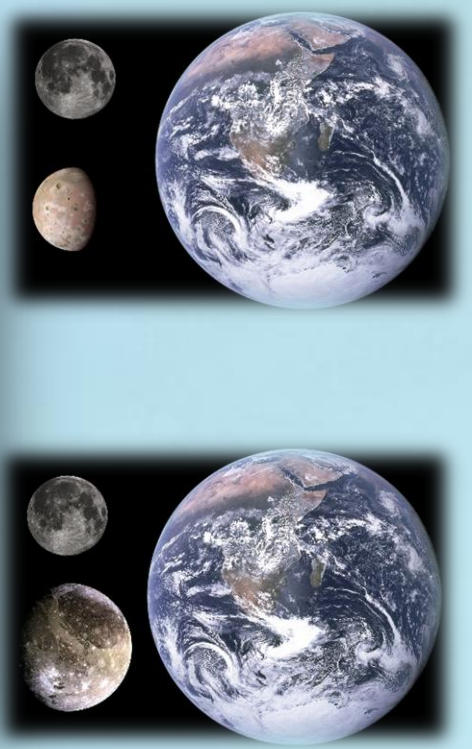
RMA of New York



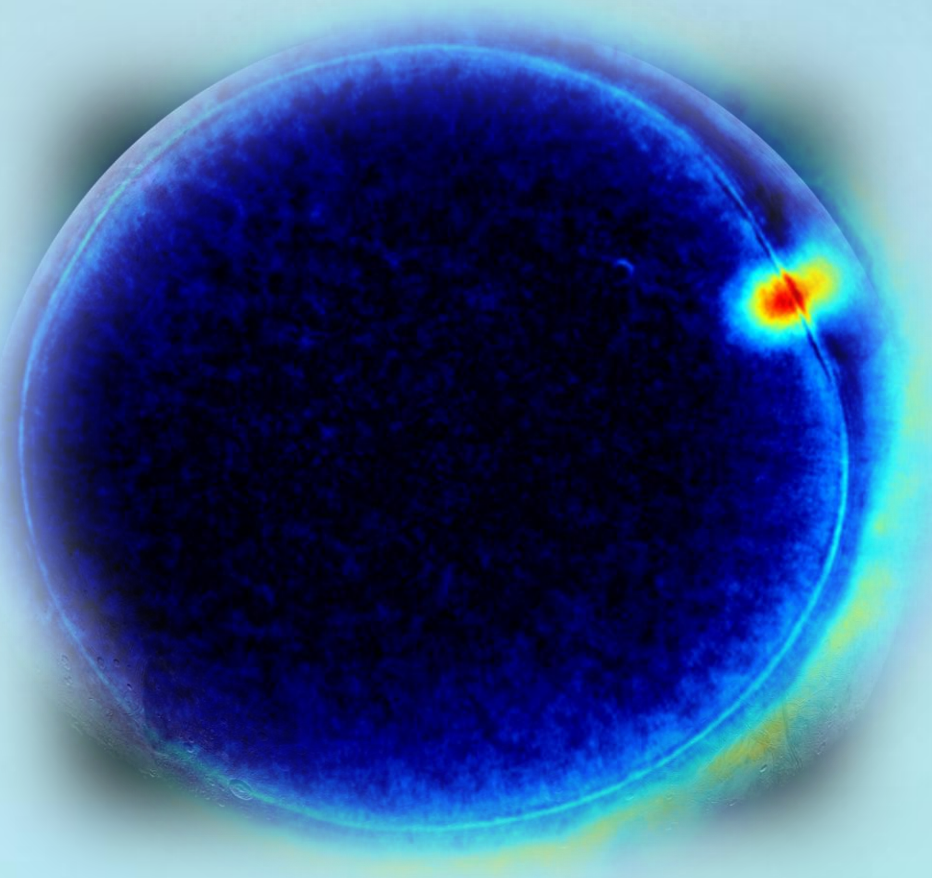
1. Io is the innermost and third-largest of Jupiter's four Galilean moons.
2. It is the most volcanically active world in the Solar System, with hundreds of volcanoes.
3. Io's thin atmosphere is primarily sulfur dioxide.
4. Discovered by Galileo Galilei in 1610, Io has a radius of about 1,131 miles.
5. The volcanism on Io is due to tidal heating from Jupiter's gravitational pull.

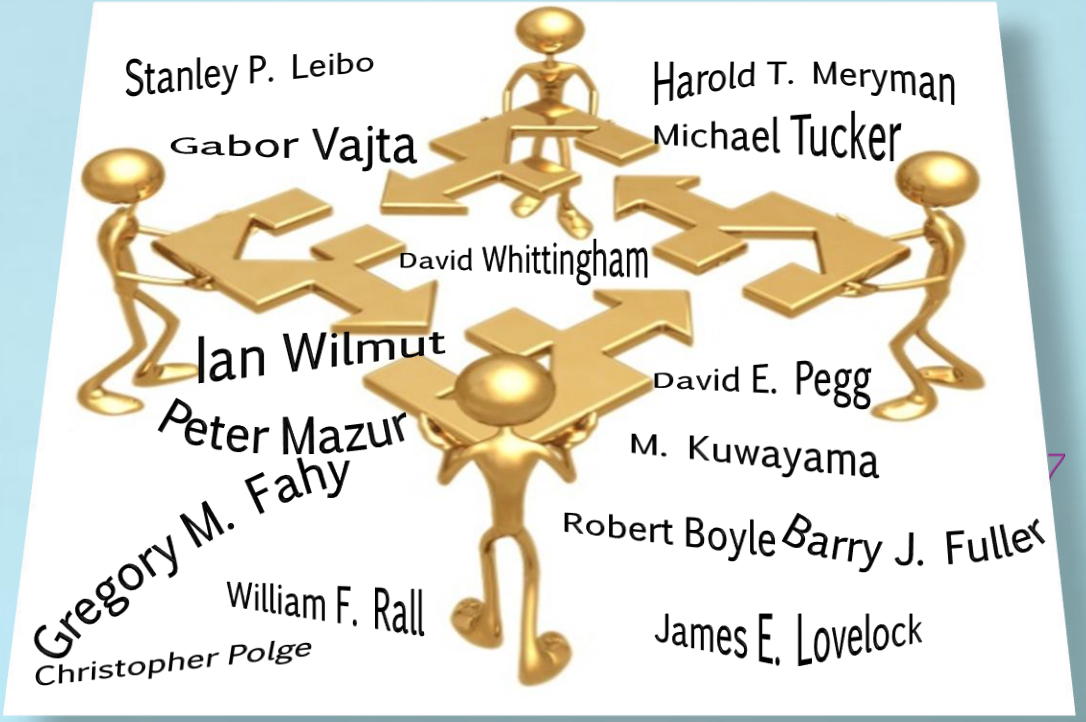
two of the four Jupiter moons

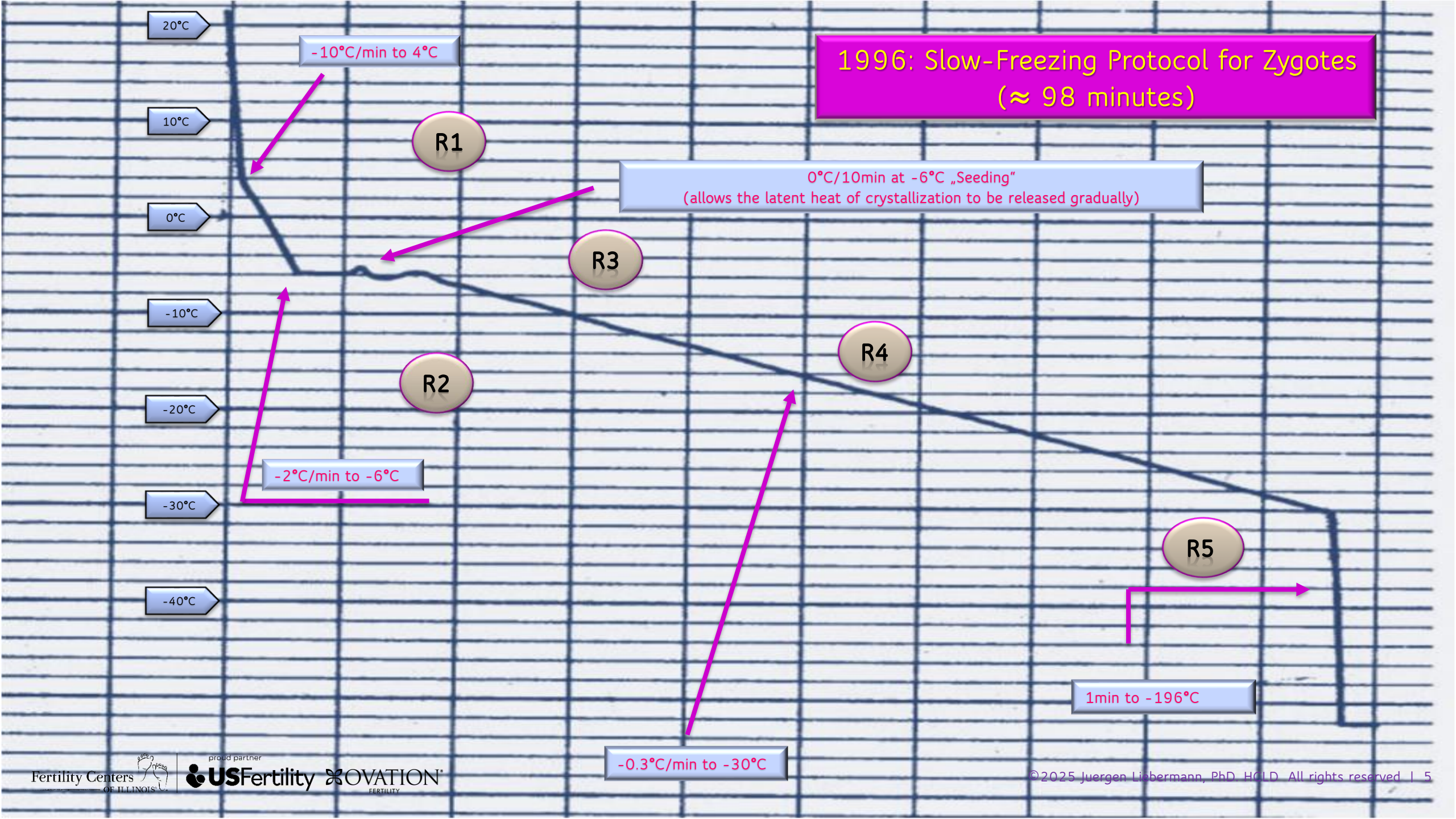
1. Ganymede is the largest moon in our solar system, bigger than Mercury and Pluto.
2. It is composed of silicate rock and water in equal proportions.
3. Ganymede has an underground saltwater ocean, potentially containing more water than all of Earth's oceans combined.
4. It is the only moon with its own magnetic field.
5. Named after a Trojan prince in Greek mythology.

Blastocyst

Oocyte



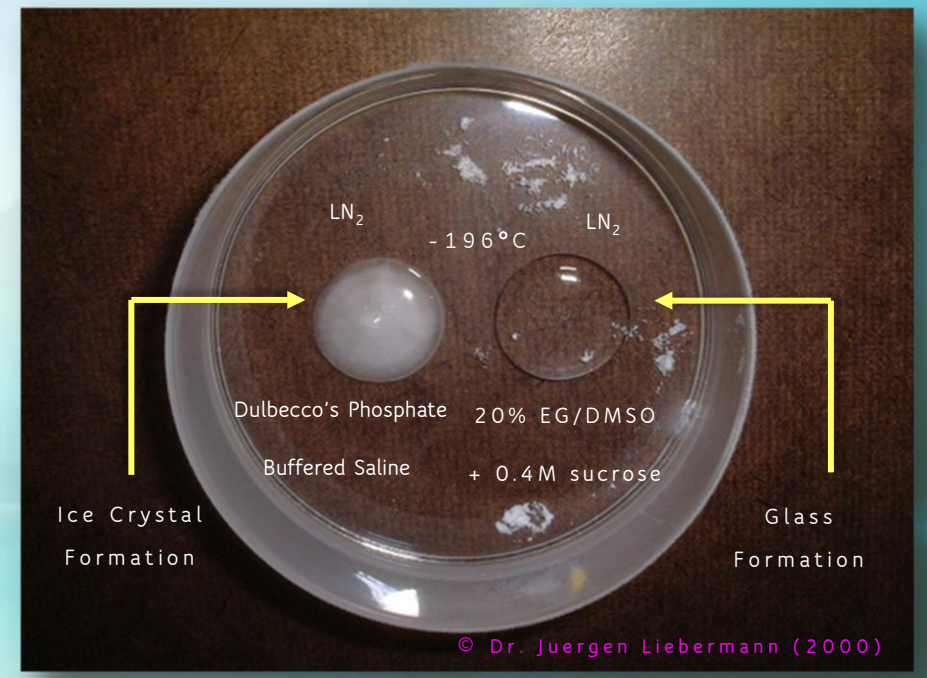




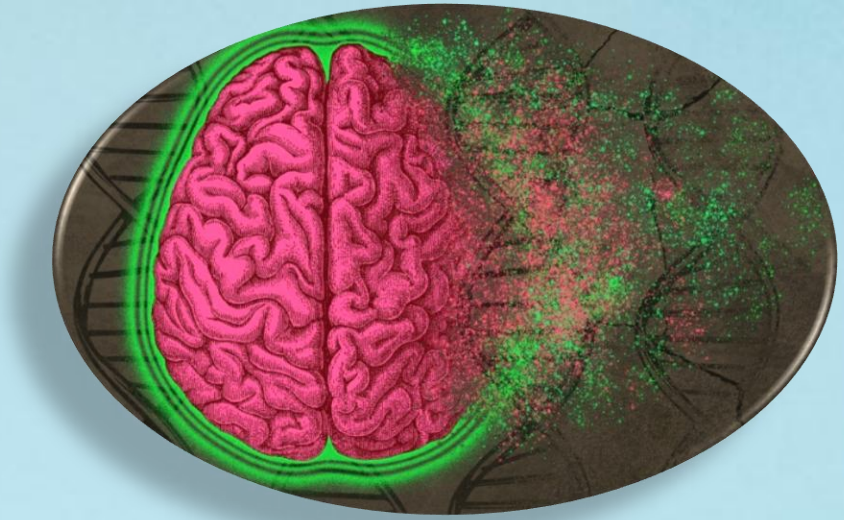
ICE CRYSTALS

should never be allowed to appear and grow inside the cells or tissues in cryopreservation procedure

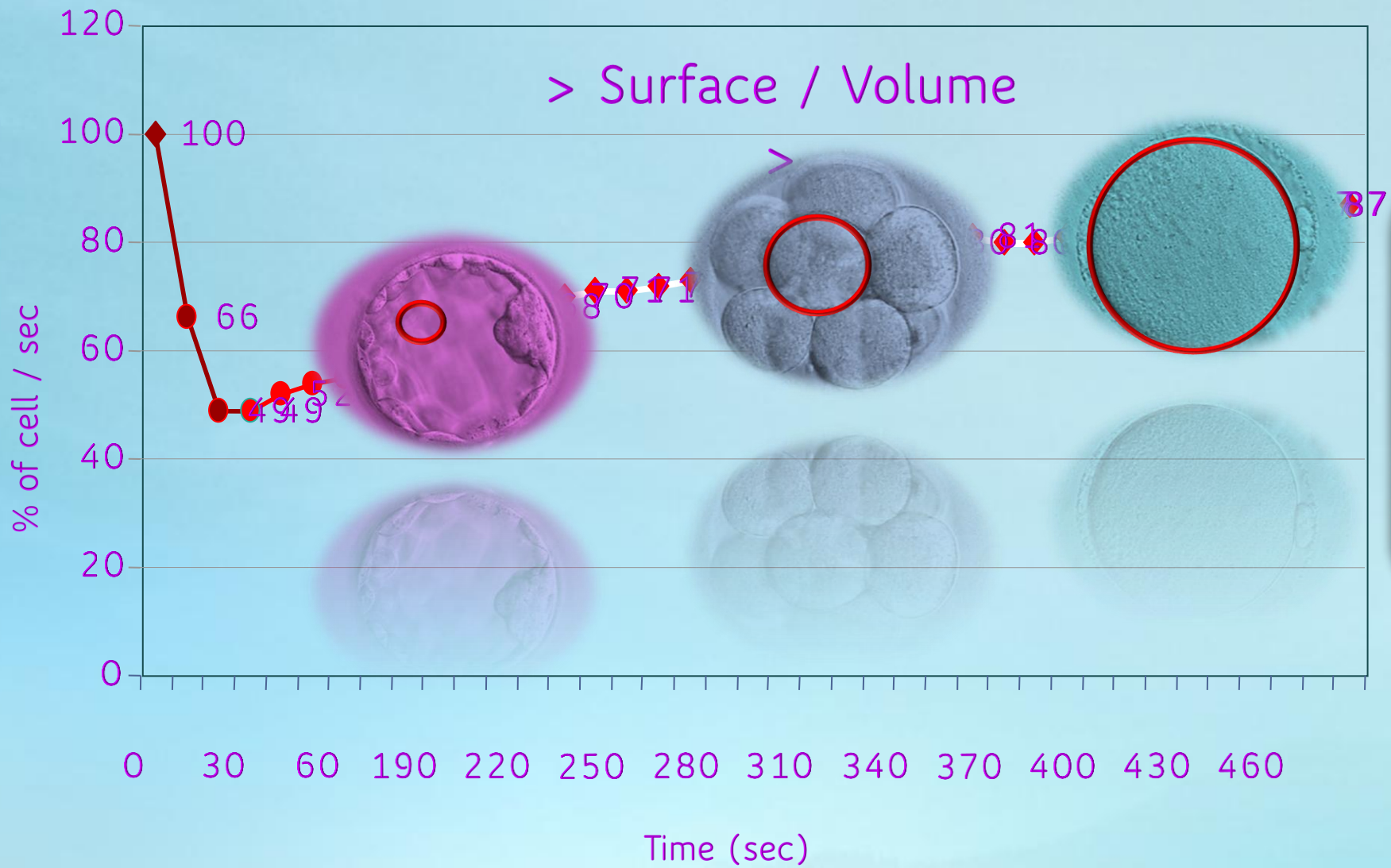
MAIN PRINCIPLE OF CRYO P R E S E R VATION



- 20 years of successful oocyte and embryo vitrification
- There are opportunities to consider that positive changes may still be possible with vitrification.
- Specifically, recent advancements have focused on the shortening and simplification of existing vitrification protocols
- A good understanding of the underlying physical, chemical and biological aspects of vitrification is crucial to understand and discern the potential of these new procedures in the ongoing improvement of cryopreservation of human gametes and embryos

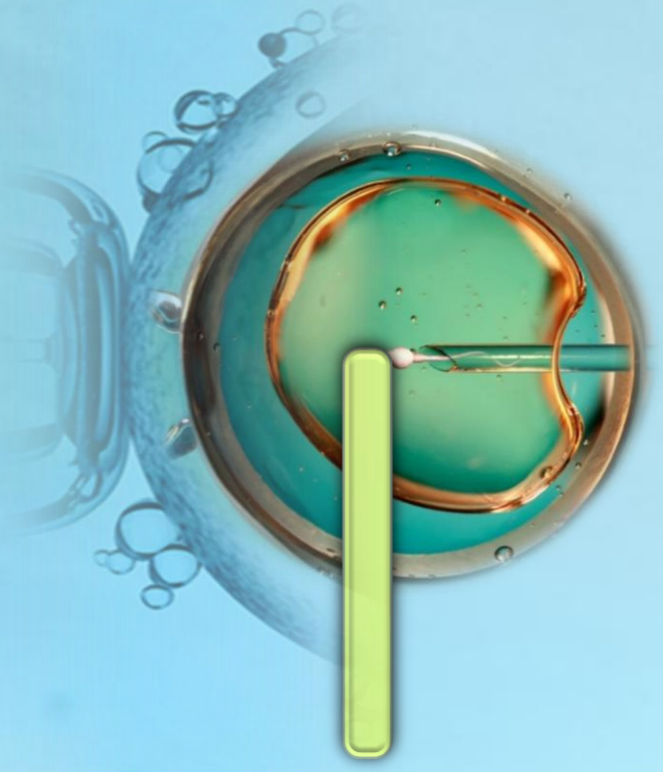


Optimal Dehydration: Surface / Volume ratio



Largest cells such as oocytes or zygotes have a low surface area to volume ratio, hence they are less efficient at taking up CP and at loosing water

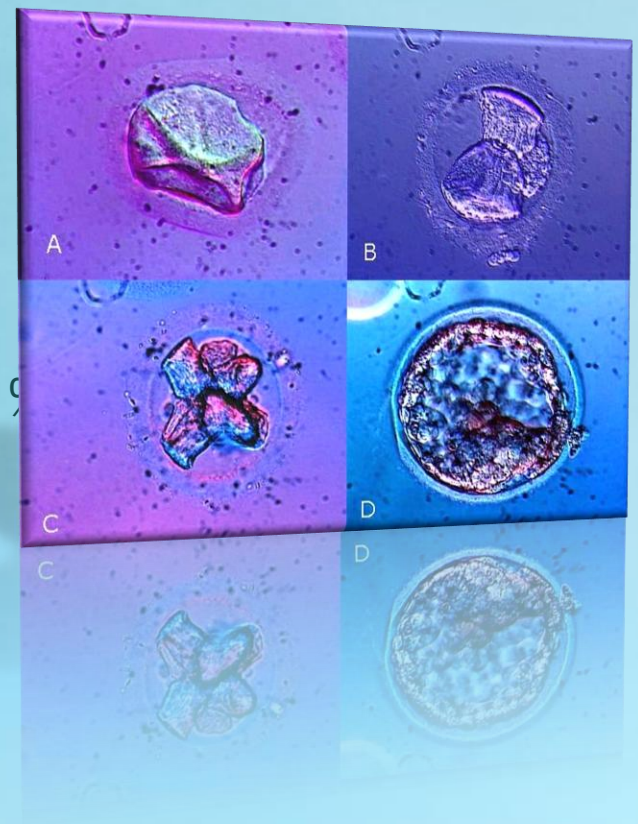
Key Factors for successful Vitrification

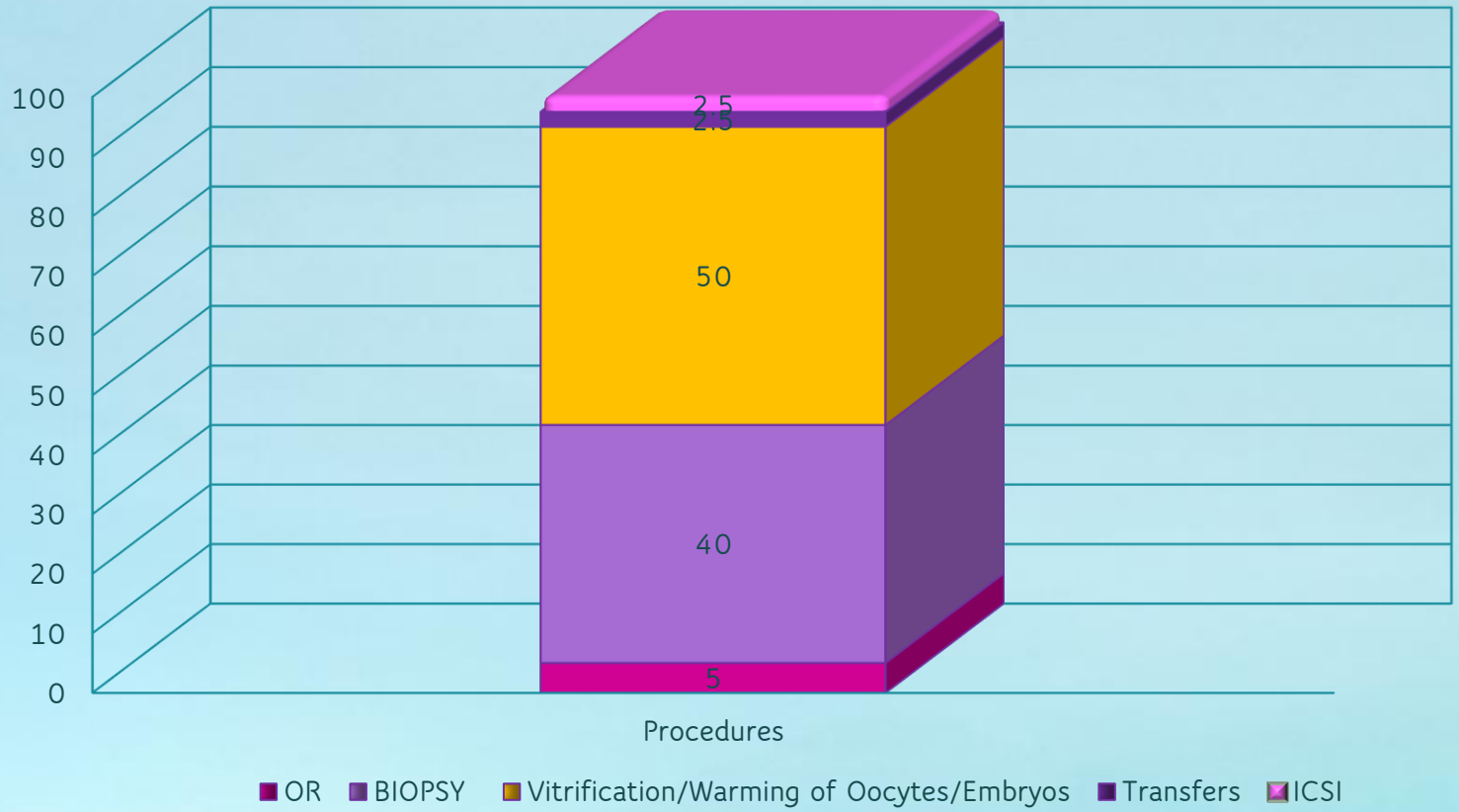


- High speed
- Short time of cooling
- Short time of warming
- Small sample volume

Low Probability of Ice Crystallization

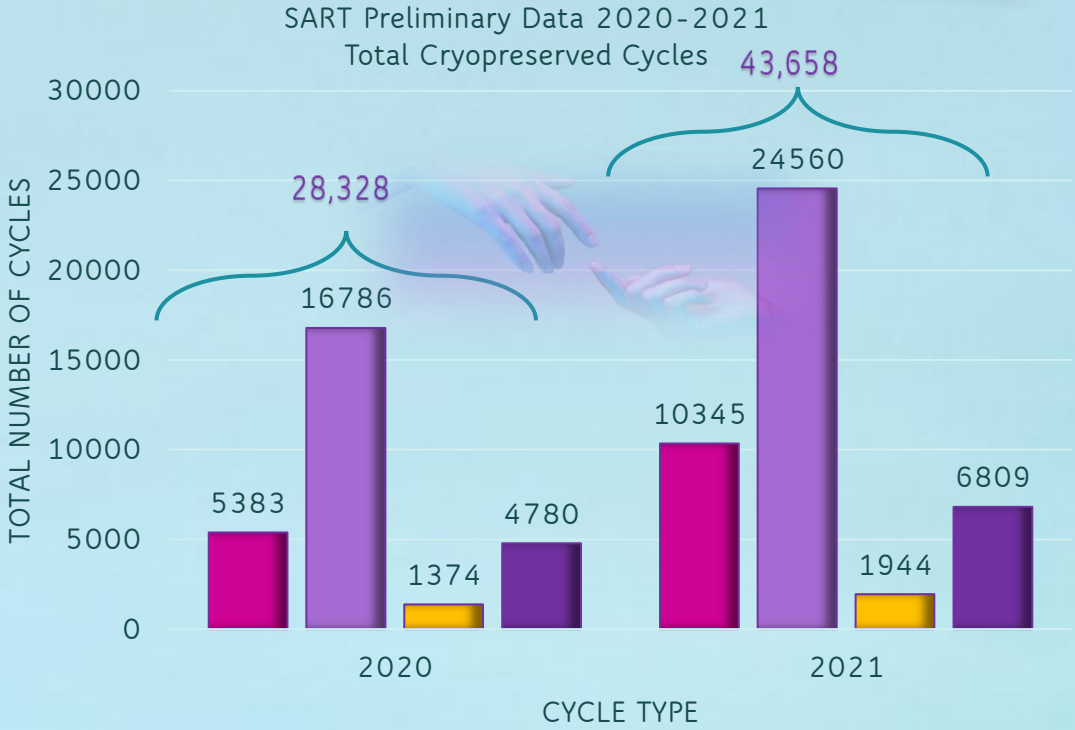
% Distribution of Daily IVF Laboratory Procedures





SART Preliminary Data 2021

- Estimated needs anticipate a 10-fold increase in IVF cycles to meet US fertility demands (1 out of 6 couples struggle with infertility)
- Total cycles 2020 vs 2021: 301,316 vs. 368,502 = 67,186 cycles more in one year; representing a 22.3% increase from 2020 to 2021.
- Total cryopreserved cycles increased from 28,328 in 2020 to 43,658, a growth of 15,330 freeze cycles
- Total number of thawed cycles using own increased from 57,700 in 2020 to 71,965 in 2021, which is a growth of 14,265 thaw cycles
- For donor eggs/embryos & donated embryos thawed cycles increased from 17,962 in 2020 to 21,897 in 2021, which is a growth of 3,935 thaw cycles
- Overall, there was an increase of close to 20,000 thawed cycles in one year
- About 30% (3.6Mill/12.0Mill) of all offspring born worldwide from IVF cycles are from oocyte and embryo cryopreservation (39th Annual Meeting of ESHRE, Copenhagen, 2023).
- On April 18th SART released their 2022 Preliminary National Data also comparing 2021 vs 2022. There are a couple of takeaways: increase of 21% in egg freezing cycles (from 24,560 to 29,803)



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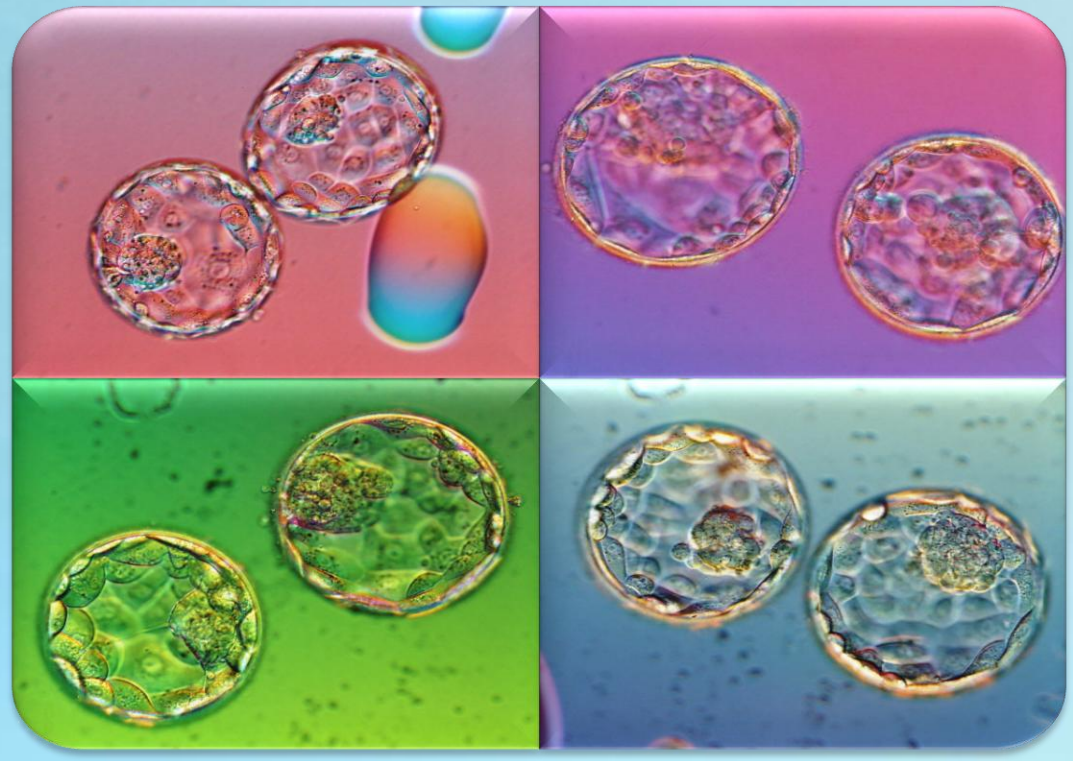


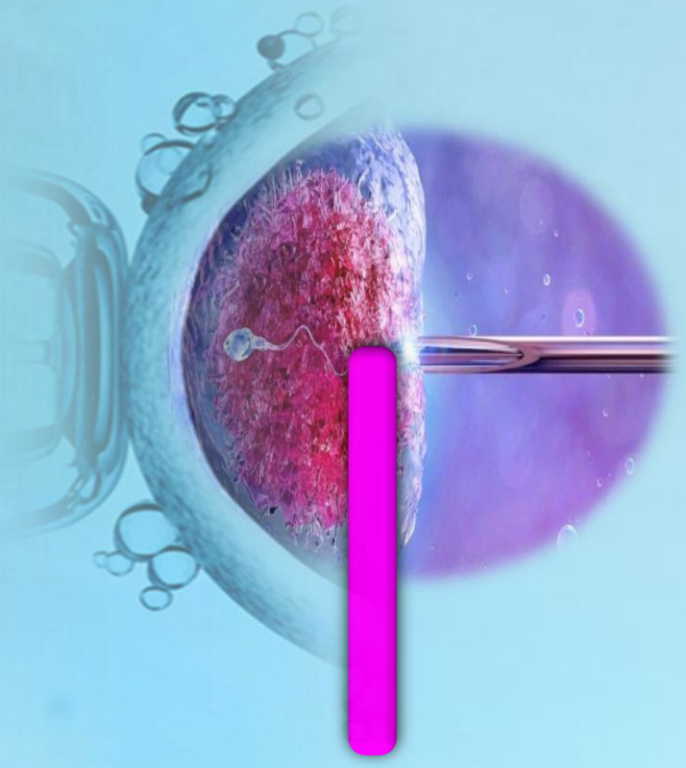
Fast and furious: pregnancy outcome with one-step rehydration in the warming protocol for human blastocysts

J Liebermann, *K Hrvojevic, J Hirshfeld-Cytron, R Brohammer, Y Wagner, A Susralski, S Jasulaitis, S Chan, E Takhsh, ML Uhler*

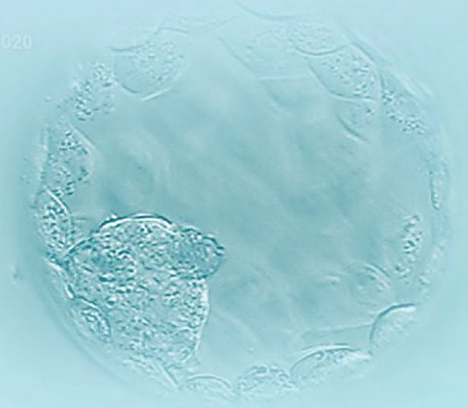
"A one-step rehydration in the warming protocol for blastocysts showed consistent and similar high survival rates with higher ongoing pregnancy and lower miscarriage rates when compared to the multi-step standard protocol. Reducing the rehydration time can contribute to improved outcomes with an efficient workflow in the IVF laboratory."

RBMO
REPRODUCTIVE BIOMEDICINE ONLINE
doi.org/10.1016/j.rbmo.2023.103731

What is known so far:



- Fundamental principle/approach in cryobiology: A stepwise removal of CP at rehydration in serial osmotic solutions
- The theory obviously behind this approach is to rehydrate the embryo by minimizing damage to the cell/embryo
- Oocyte/Embryo vitrification/warming are time-consuming and labor-intensive

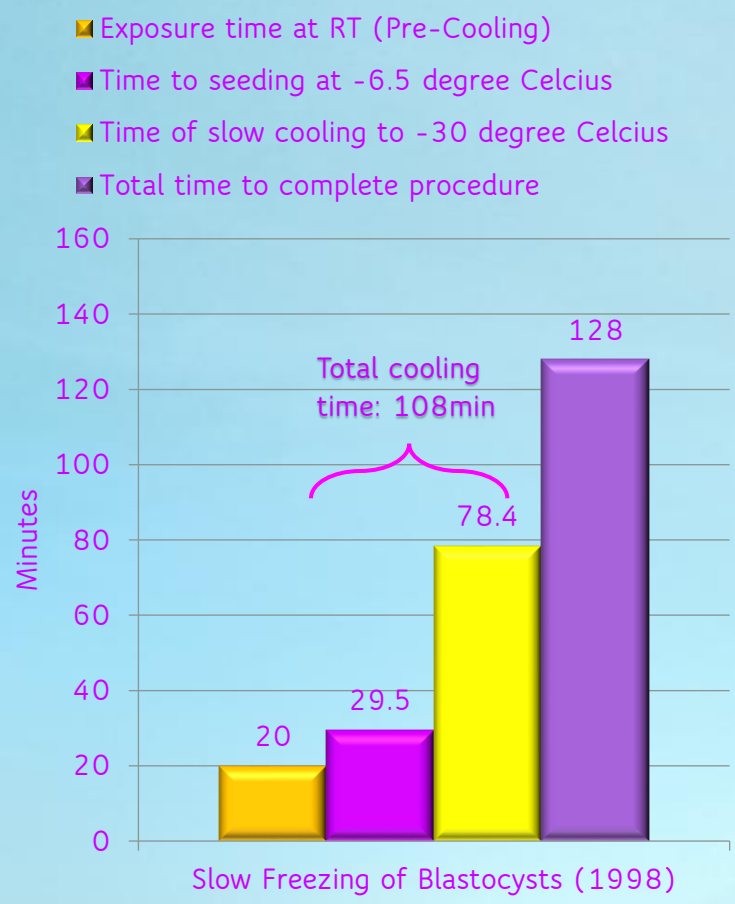


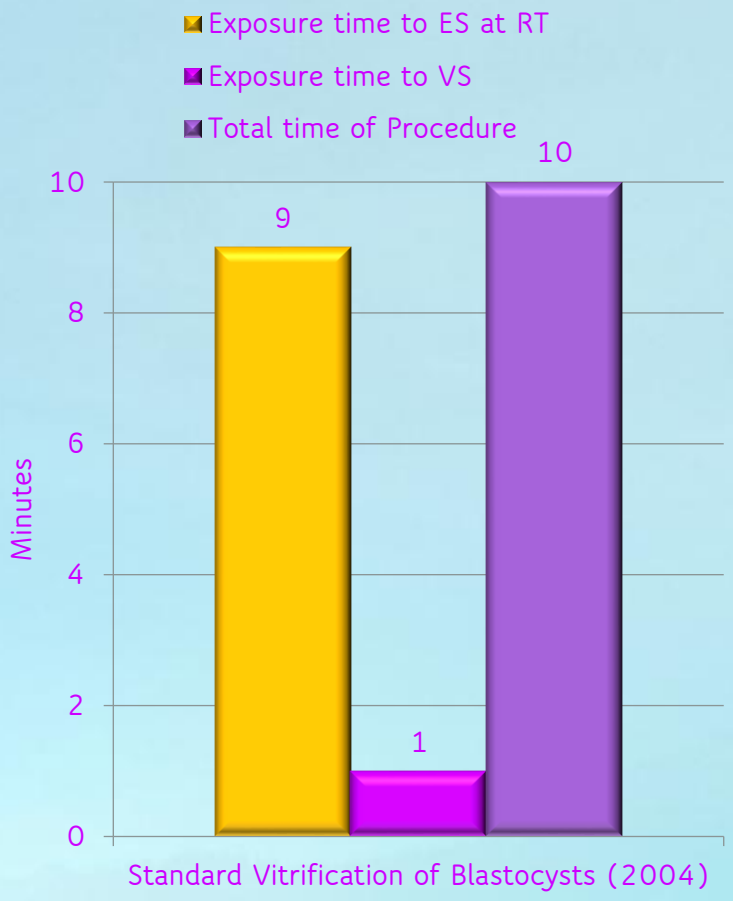
- Having a way to achieve 90-100% survival using a single step during rehydration would challenge the dogma of cryobiology

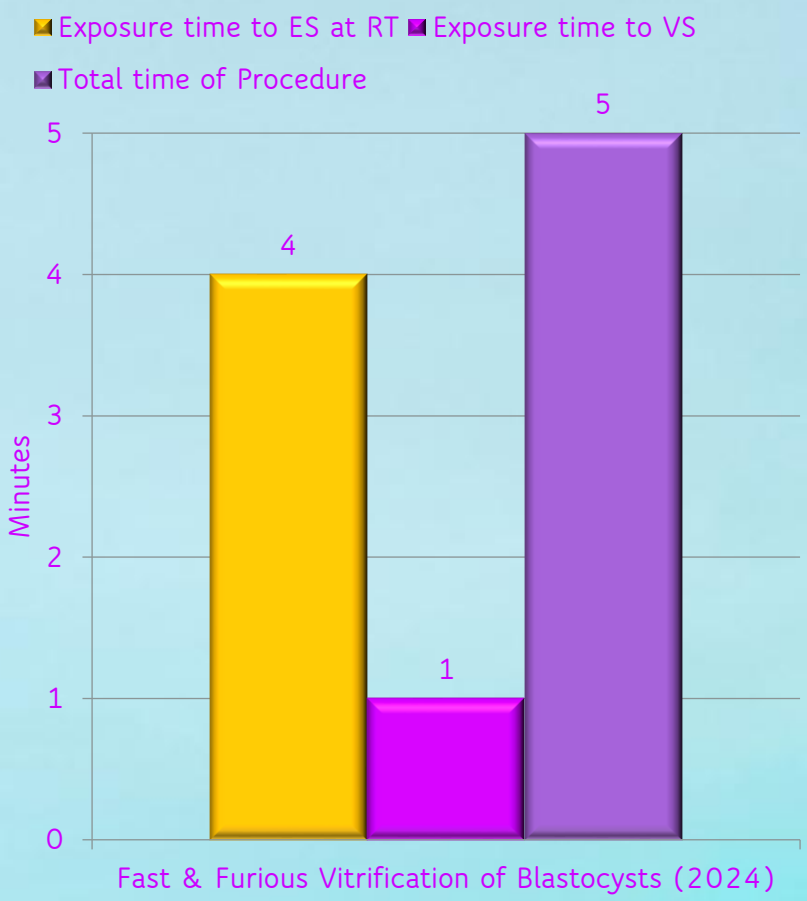
Development of Blastocyst Cryopreservation covering 26 years

92% time saving for one Vit

96% time saving for one Vit







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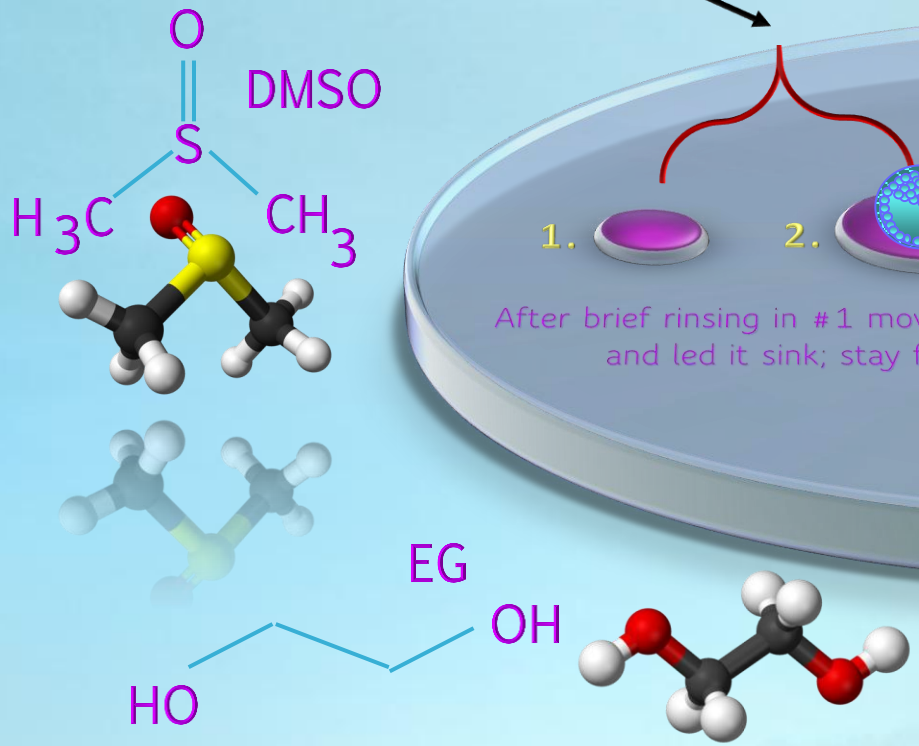
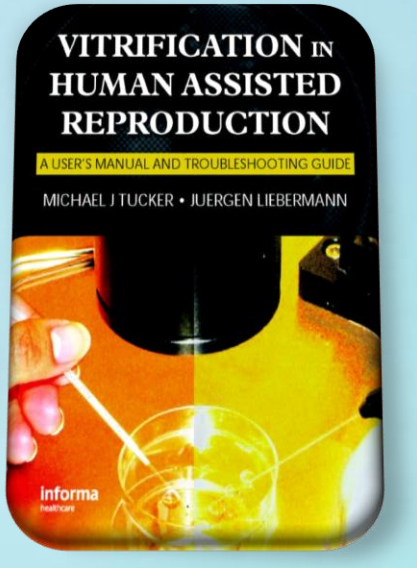
Modified Blastocyst Vitrification Procedure on a plain 90mm Dish Lid Surface (takes 5min)

At RT | 2x drops of ES (50 &100µl - total of 4mins)



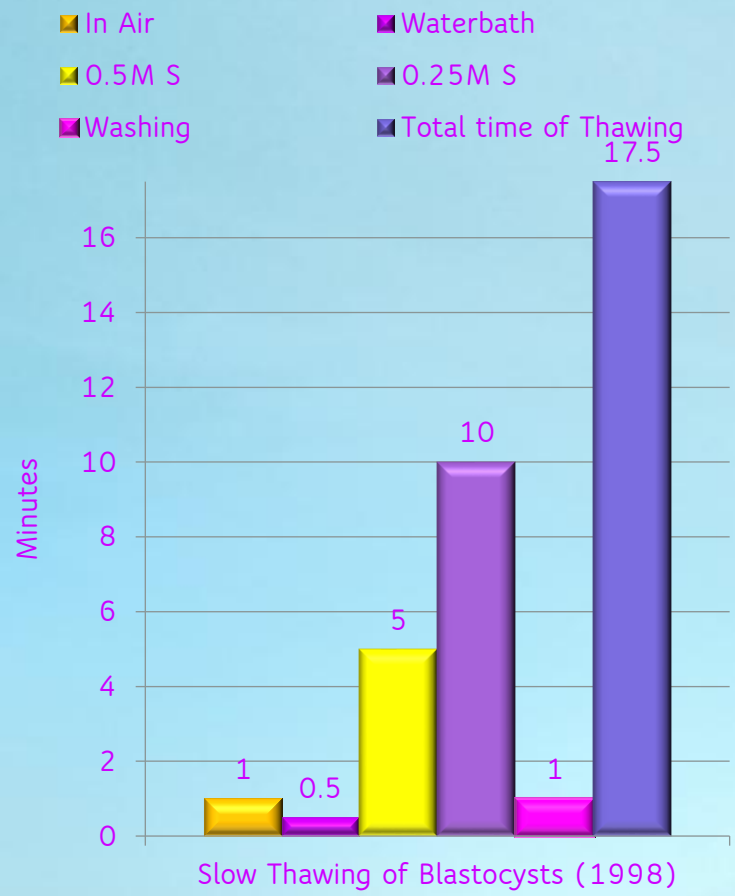


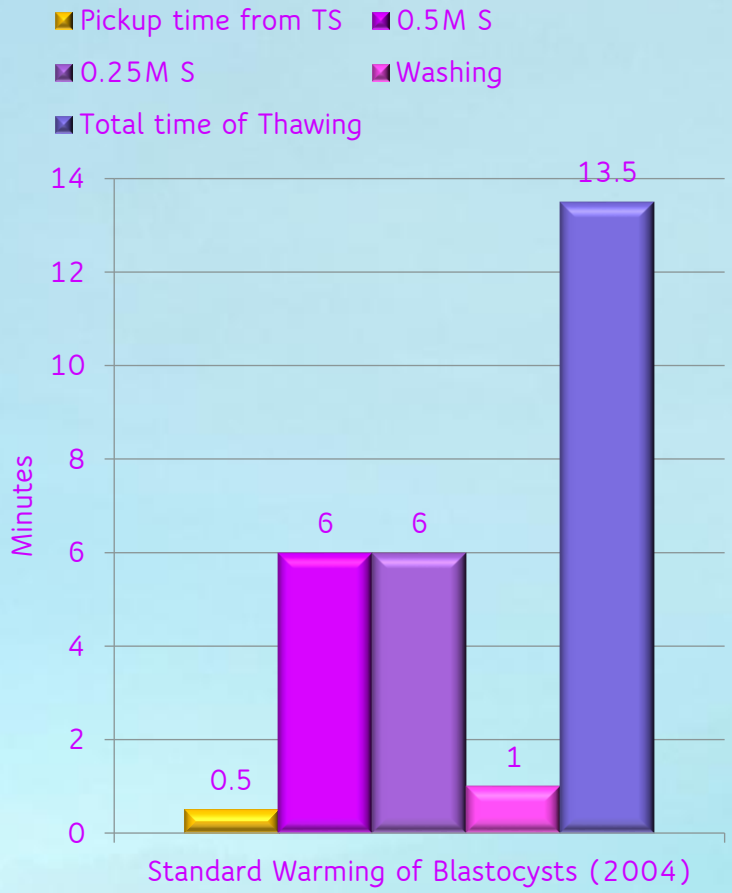
At RT | VS (2x100µl - 1min)

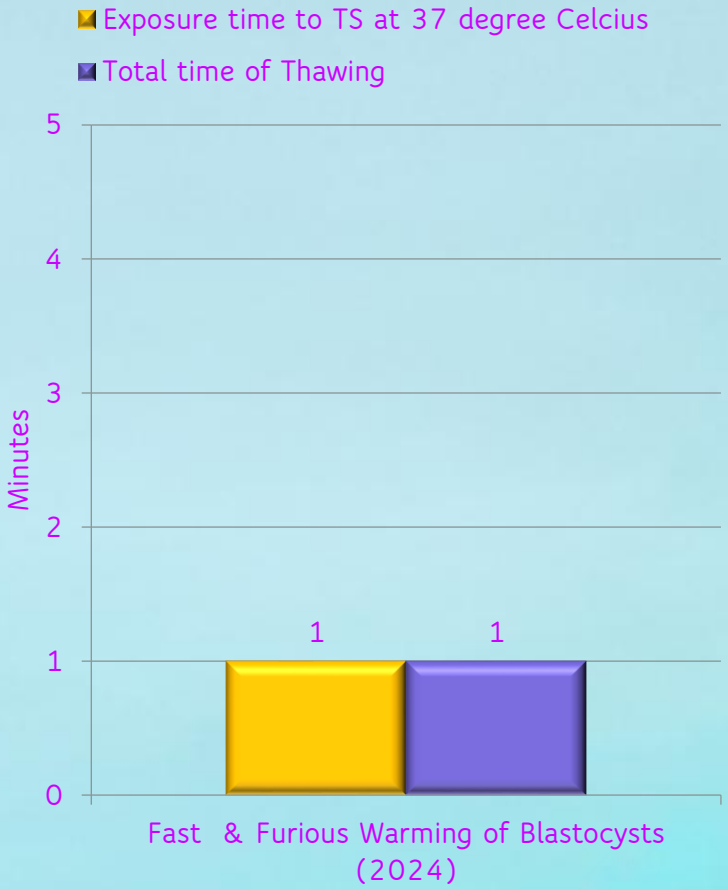
Development of Blastocyst Thawing covering 26 years

28.5% time saving
for one Warming

94% time saving
for one Warming







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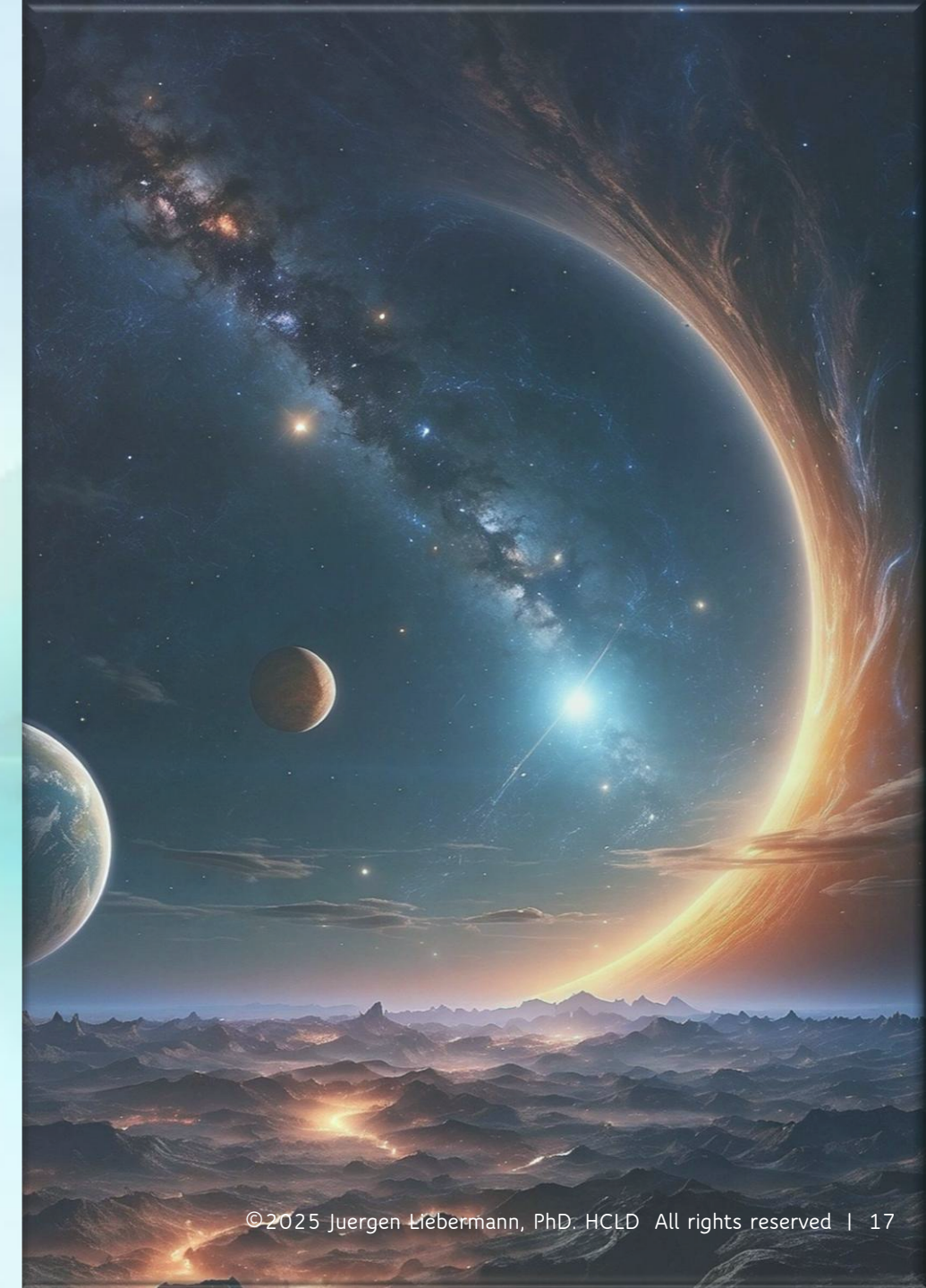
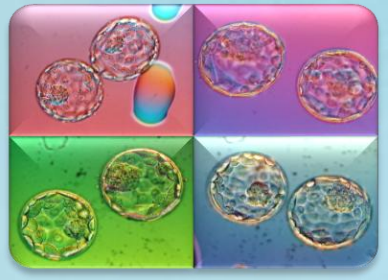
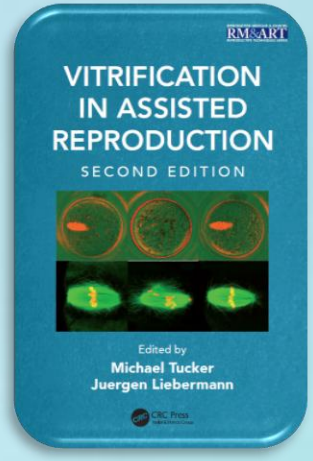
Recognizing the Research and Publications on 1-step warming by Stanley Leibo (1983); El-Gayar *et al.* (2001) and recently by Manns *et al.*; 2021; 2022 & Taylor *et al 2022* [Prize paper at PCRS 2022 & Abstracts at ASRM





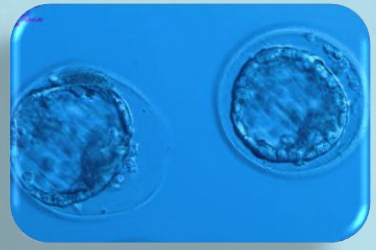
Results from a 1min *warming* in 1M TS from donated embryos for research divided into biopsied and not biopsied day 5, day 6 or day 7 blastocysts

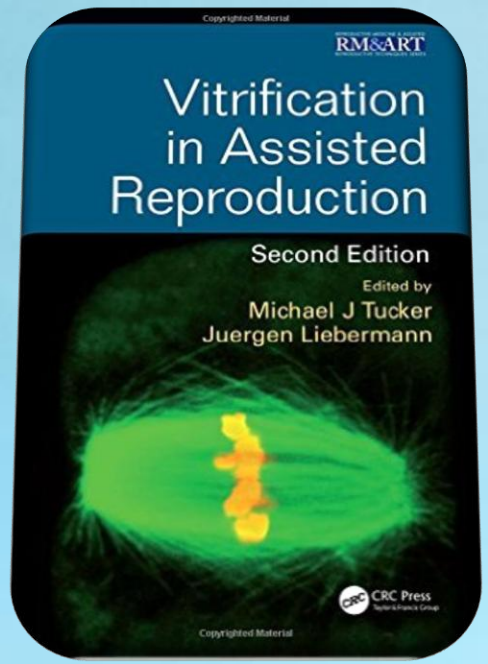
N (Warmed)	Survival Post warm	Survival after 24 hrs	#Hatching/hatched out after 24 hrs
666	641 (96.2%)	612 (91.9%)	474 (77.5%)
N (biopsied)			
386	377 (97.7%)	352 (91.2%)	307 (87.2%)
N (Not biopsied)			
280	264 (94.3%)	260 (92.8%)	167 (64.2%)



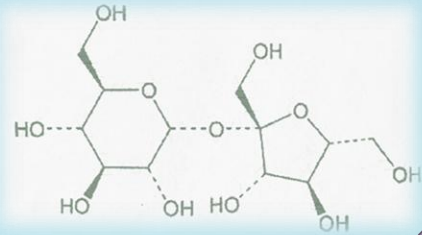
Trial started in May of 2022; Implemented in clinic setting on September 22nd, 2022

Modified Blastocyst Warming Procedure on a plain 90mm Dish Lid Surface (takes 1min)





1min in 400µl TS (37°C) on a 90mm Dish Lid surface, then straight to the transfer dish

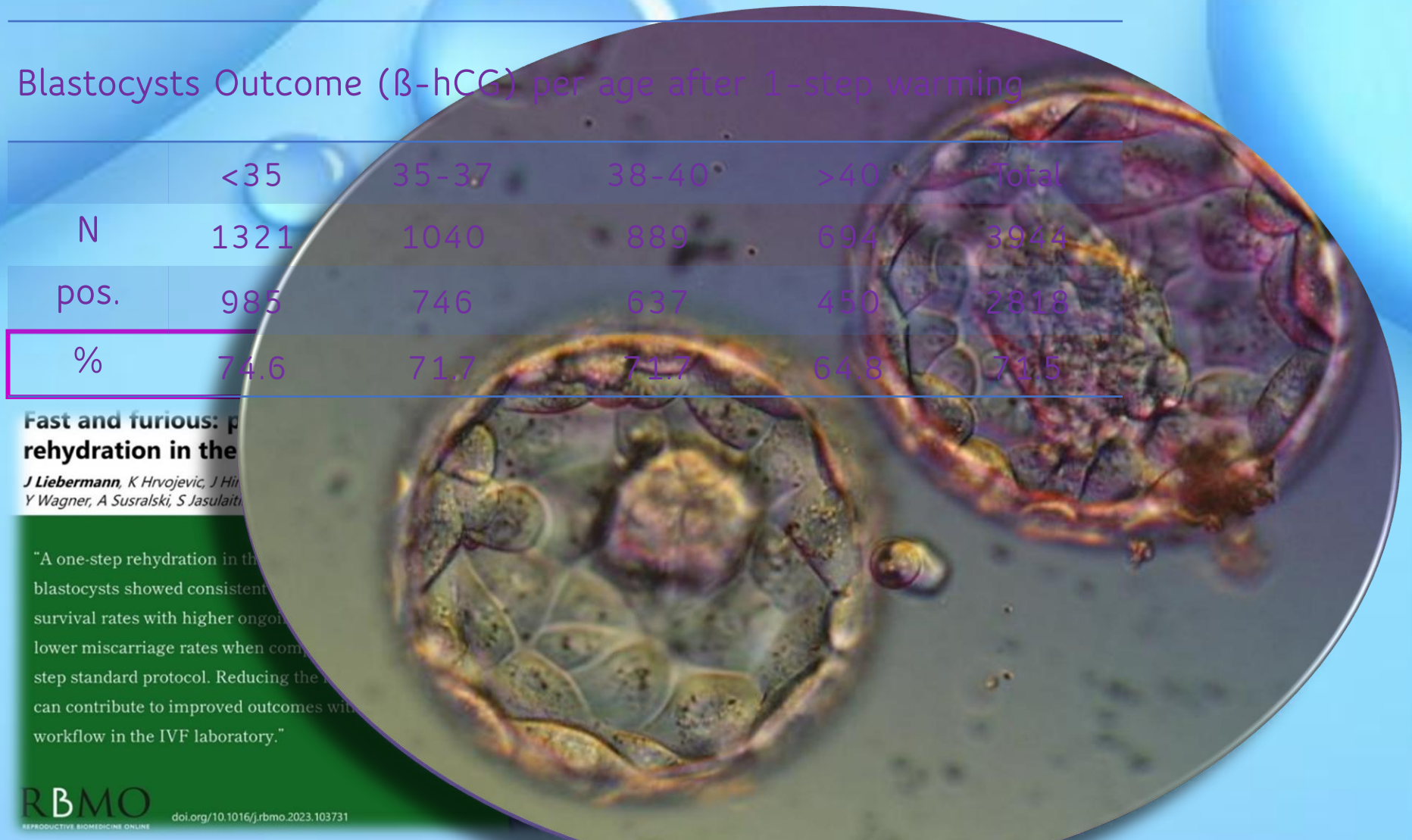


Transfer dish with SBM and 20% SPS

Blastocyst Outcome (day 5-7) per age after a one-step rapid warming protocol in 1M Sucrose for 1min from 9/22/2022 - 12/06/2024 (n=3741)

Blastocysts Outcome (ß-hCG) per age after 1-step warming

	<35	35-37	38-40	>40	Total
N	1321	1040	889	694	3944
pos.	985	746	637	450	2818
%	74.6	71.7	71.7	64.8	71.5



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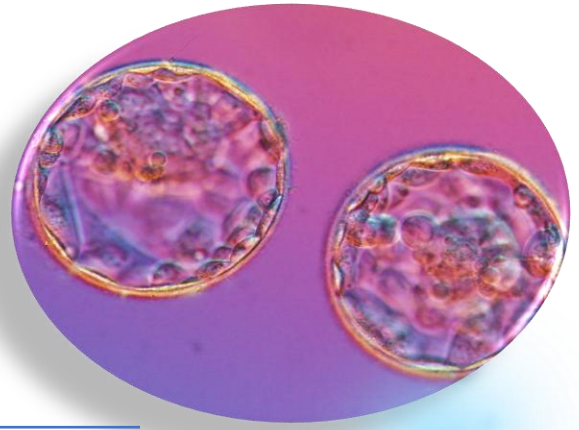
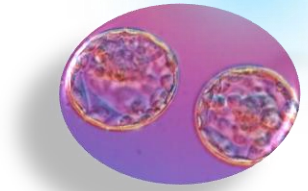
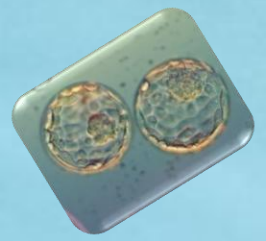
High Volume Procedure Days in December 2024 and FET outcome



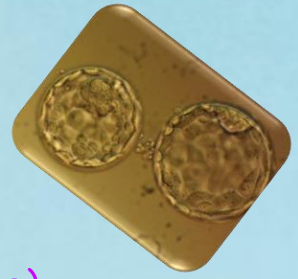
High Volume Procedure Days in December 2024

Procedure/ Day	12/12	12/13	12/16	12/17	12/18	Total
n OR	8	12	15	20	5	60
n FETs	16	15	17	11	11	70
n Total	24	27	32	31	16	130
Average Age FETs	36.3 ± 4.0	37.6 ± 3.1	35.2 ± 4.3	36.5 ± 5.3	35.4 ± 3.5	36.2 ± 4.0
n FETs Positive beta	11	11	13	9	9	53
% Positive beta	68.8	73.3	76.5	81.8	81.8	75.7





Live Births from rapid warming (takes 1min) of vitrified Blastocysts (all ages, all embryos, untested vs tested, all days of developments)



All embryos, all ages)

All Embryos	
No. of Transfers	2070
Avg Age	36.6±4.5
Positive PR (%)	71.8
cPR (%)	61.7
oPR (%)	51.7
IR (%)	60.4
N Babies born	1092
N Live Births per CDC	1069
Live Birth (%) per CDC	51.6
Avg Weight (g)	3265±661
Avg PR Duration (weeks)	37.6±2.4
Male (%)	49.0
Female (%)	51.0
Singleton (%)	98.0
Twins (%)	1.8
Triplets (%)	0.2

CDC defines live birth as one regardless of number of infants born

Untested embryos, all ages)

Untested Embryos	
No. of Transfers	763
Avg Age	35.9±4.8
Positive PR (%)	69.1
cPR (%)	58.5
oPR (%)	46.9
IR (%)	56.0
N Babies born	373
N Live Births per CDC	357
Live Birth (%) per CDC	46.8
Avg Weight (g)	3224±720
Avg PR Duration (weeks)	37.0±3.0
Male (%)	52.5
Female (%)	47.5
Singleton (%)	96.0
Twins (%)	3.4
Triplets (%)	0.6

Euploid embryos, all ages)

Euploid Embryos	
No. of Transfers	1307
Avg Age	37.0±4.3
Positive PR (%)	73.4
cPR (%)	63.7
oPR (%)	54.5
IR (%)	63.2
N Babies born	719
N Live Births per CDC	712
Live Birth (%) per CDC	54.5
Avg Weight (g)	3265±661
Avg PR Duration (weeks)	37.7±2.1
Male (%)	47.1
Female (%)	52.9
Singleton (%)	99.0
Twins (%)	1.0
Triplets (%)	0.0

1092 LIVE BIRTHS

SART defines ongoing pregnancy as one regardless of number of GSs seen on imaging ("Multiple gestational sacs in one patient are counted as ONE clinical pregnancy"),

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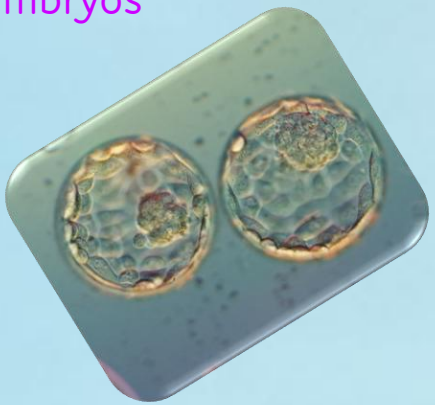
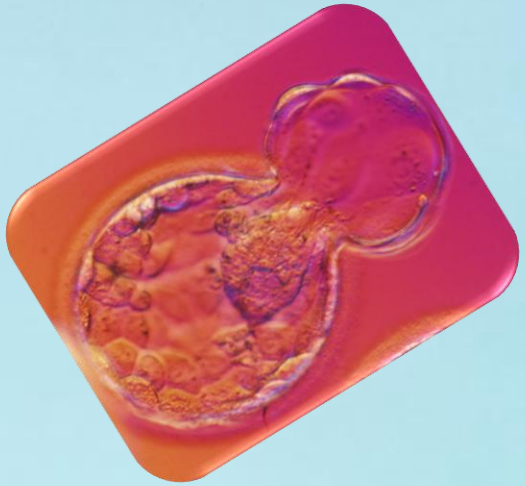
Live Births from rapid warming (takes 1min) of vitrified Blastocysts for Patients <35 and Patients >40 (all FETs)

All Embryos <35	Patients <35; all embryos
No. of Transfers	675
n FET untested	317
% FET untested	47.0
n FET euploid	358
% FET euploid	53.0
Positive PR (%)	73.8
cPR (%)	64.4
oPR (%)	57.0
IR (%)	63.4
N Babies born	396
N Live Births per CDC	384
Live Birth (%) per CDC	56.9
Avg Weight (g)	3255±688
Avg PR Duration (weeks)	37.0±2.5
Male (%)	48.7
Female (%)	51.3
Singleton (%)	97.1
Twins (%)	2.6
Triplets (%)	0.3



All Embryos >40	Patients >40; all embryos
No. of Transfers	368
n FET untested	109
% FET untested	29.6
n FET euploid	259
% FET euploid	70.4
Positive PR (%)	65.5
cPR (%)	54.1
oPR (%)	44.8
IR (%)	52.3
N Babies born	163
N Live Births per CDC	163
Live Births (%) per CDC	44.3
Avg Weight (g)	3223±624
Avg PR Duration (weeks)	37.0±2.0
Male (%)	42.3
Female (%)	57.7
Singleton (%)	100.0
Twins (%)	0
Triplets (%)	0



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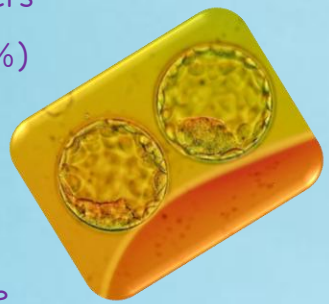
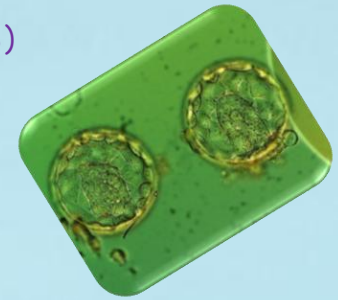
Live Births from rapid warming (takes 1min) of vitrified Blastocysts for Patients <35 and Patients >40 (untested vs euploid FETs)

Patients <35; Untested vs euploid embryos

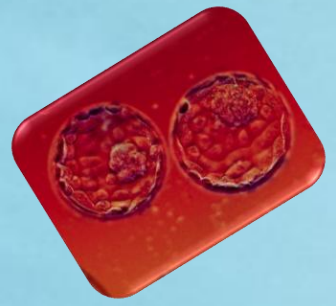


Patients <35	Untested Embryos	Euploid Embryos
No. of Transfers	317	358
Positive PR (%)	71.9	75.4
cPR (%)	61.2	67.3
oPR (%)	53.3	60.3
IR (%)	59.8	66.8
% Miscarriage	13.9	10.4
N Babies born	174	222
N Live Births per CDC	168	216
Live Births (%) per CDC	53.0	60.3
Avg Weight (g)	3259±682	3252±699
Avg PR Duration (weeks)	38.0±3.5	38.0±2.0
Male (%)	49.4	48.2
Female (%)	50.6	51.8
Singleton (%)	97.0	97.2
Twins (%)	2.4	2.8
Triplets (%)	0.6	0

Patients >40; Untested vs euploid embryos



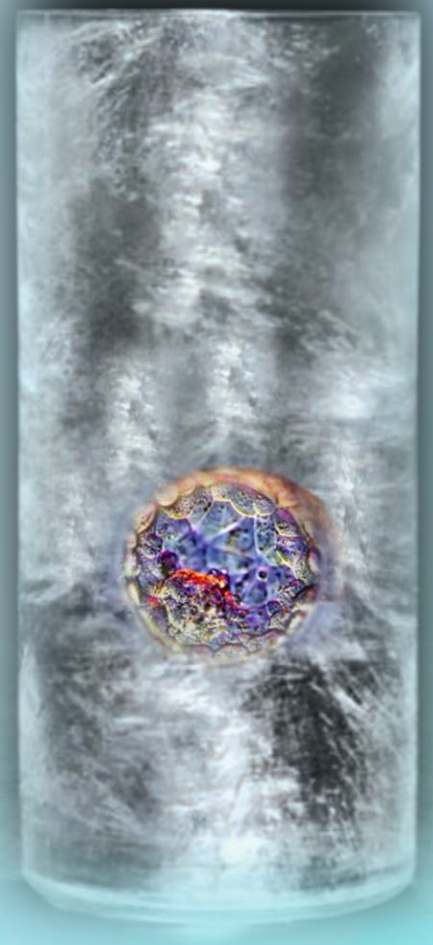
Patients >40	Untested Embryos	Euploid Embryos
No. of Transfers	109	259
Positive PR (%)	58.7	68.3
cPR (%)	48.6	56.4
oPR (%)	35.8	48.3
IR (%)	45.2	55.7
% Miscarriage	26.4	14.4
N Babies born	39	124
N Live Births	39	124
Live Births (%)	35.8	47.9
Avg Weight (g)	3288±548	3202±644
Avg PR Duration (weeks)	37.0±2.0	37.0±2.0
Male (%)	48.7	40.3
Female (%)	51.3	59.7
Singleton (%)	100.0	100
Twins (%)	0	0
Triplets (%)	0	0



Live Births from rapid warming (takes 1min) of vitrified Blastocysts for Patients <35 (Day 5; untested vs euploid FETs)

Patients <35; Day 5 & Day 6; Untested vs euploid embryos

Patients <35	Untested Day 5	Euploid Day 5	Untested Day 6	Euploid Day 6
No. of Transfers	206	215	108	141
Positive PR (%)	72.8	80.5	70.4	68.1
cPR (%)	61.7	71.6	60.2	61.0
oPR (%)	54.4	65.1	50.9	53.2
IR (%)	61.2	71.5	57.7	59.7
% Miscarriage	11.8	9.1	15.4	12.8
N Babies born	113	145	59	76
N Live Births	111	140	55	75
Live Births (%)	53.9	65.1	50.9	53.2



To summarize:

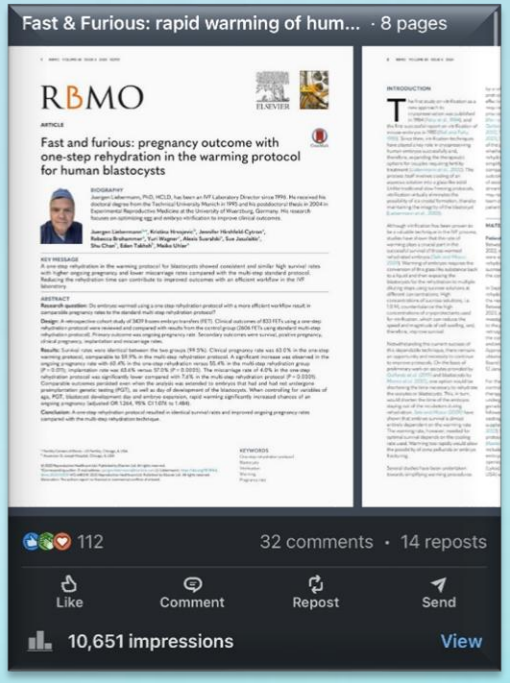
summary on short Dehydration

It makes laboratory technique for warming blastocysts simpler.

It reduces the unnecessary time blastocysts are exposed to room temperature and in turn reduces stress to the blastocysts. There is less time for Radical Oxygen Species (ROS) creation and their impact on the embryo development.

Short dehydration/short rehydration is easy to implement in your daily routine and standardizes your blastocyst cryopreservation procedure and its outcome even more successful

The one-step warming protocol is superior to the multi-step protocol in terms of pregnancy outcome, supporting an optimized workflow by reducing labor to 95%, as well as cost-effectiveness (40%) in any IVF Laboratory.



Benefits for Embryos & Embryologists:

Since implementing the fast rehydration protocol in July 2024, we've seen a significant improvement in our frozen embryo transfer outcomes. Thank you so much again for your efforts to enhance embryology practices, it has truly made a difference.

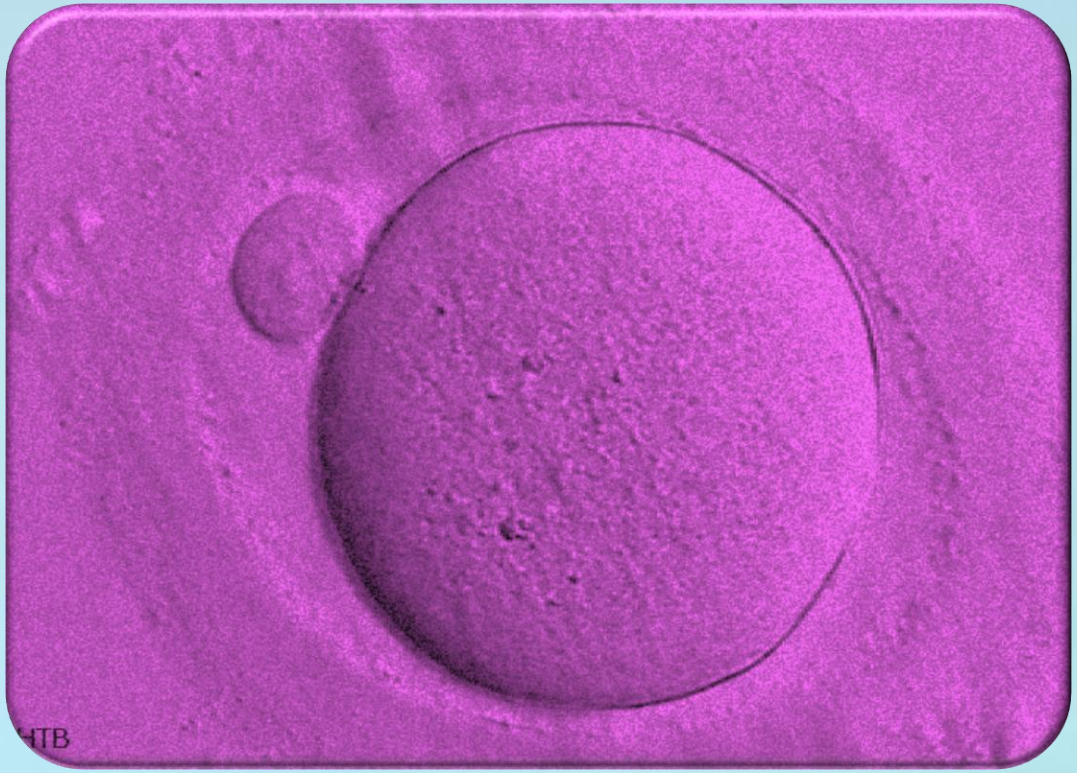
Clinical Embryology RBMO

Fast and furious: successful survival and resumption of meiosis in immature human oocytes vitrified and warmed in a short protocol

Juergen Liebermann, Rebecca Brohammer, Yuri Wagner, Ru Smith, Kelly Even, Jennifer Hirshfeld-Cytron, Meike Uhler

"Vitrification and warming of oocytes at different nuclear maturation stages can be done with a 2-minute exposure time to hypertonic and a 2-minute exposure time to hypotonic solutions. With this approach, the oocytes are less exposed to room temperature during dehydration and rehydration. Warming in 0.5M sucrose helps to maintain and support the potential to resume nuclear meiotic activity of oocytes and conversion from GV to MI and to MII oocytes."

doi.org/10.1016/j.rbmo.2024.103976



Egg Vitrification

26,115 autologous eggs from 2487 cycles (average of 11 eggs per patient with an average age of 34.6)

From about 2942 eggs warmed (average of 10 eggs per patient with an average age of 40.7) we can observe a clinical PR of 60% and an ongoing PR of 57.5% per transfer.

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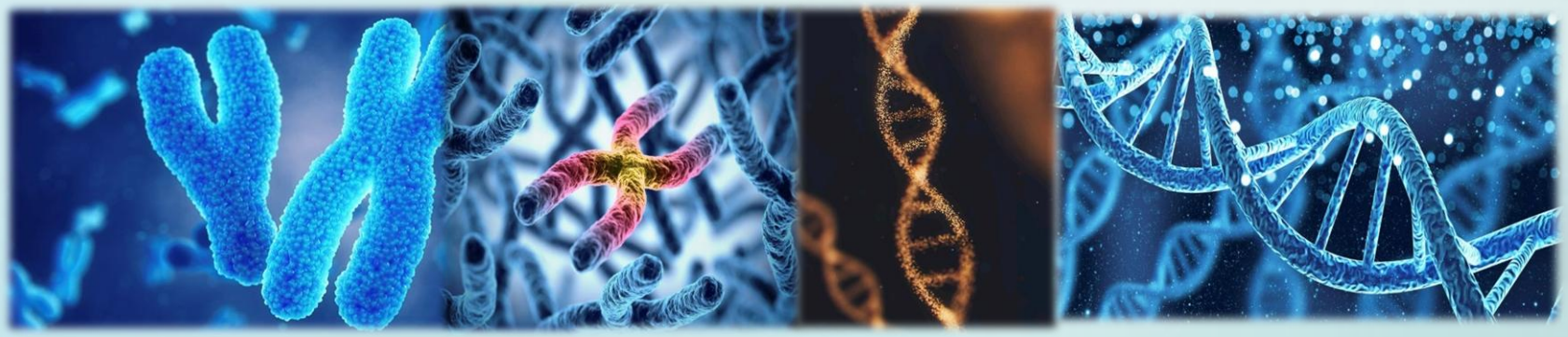
Obtained acceptance for several applications:

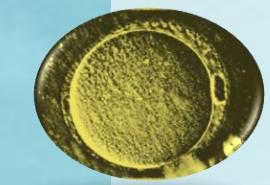
- No sperm available on day of egg retriev
- Poor responder – multiple cycles su n
- Egg donation (convenience, indepe ng)
- Fertility preservation in cancer patie
- Age-related fertility loss – social freezi
- Premature ovarian failure



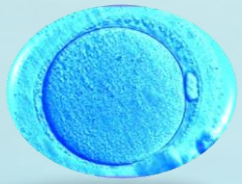
In 2024

Egg freezing is safe, data are reliable, repeatable, and successful. However .....

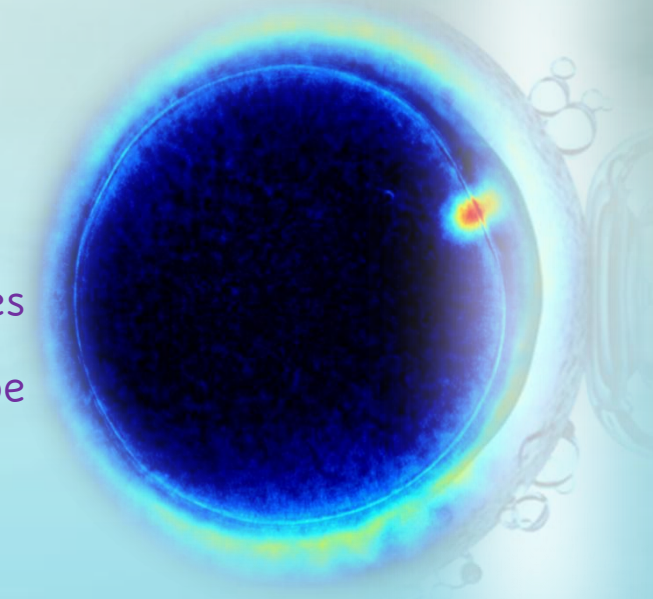
Is there a missing piece of the puzzle regarding oocyte vitrification/warming?

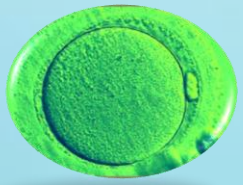


- Gallardo, Sanz & Risco, 2019 (Scientific Reports) discovered that

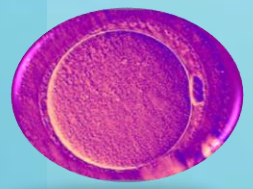


- Oocytes' osmotic behavior indicate that the dehydration upon exposure to CPA solutions occurs within 60 seconds



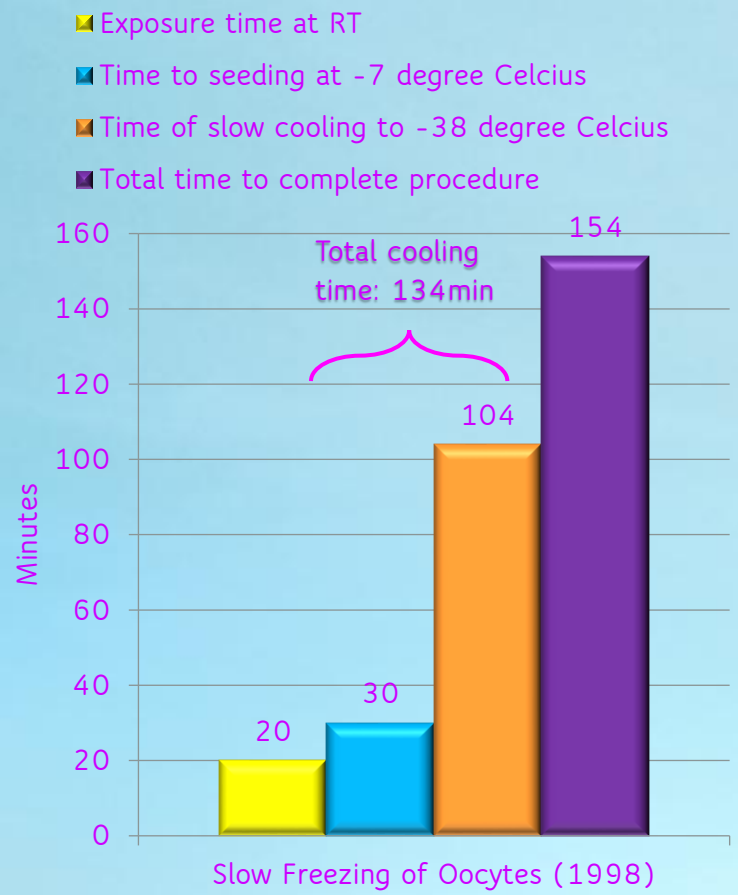


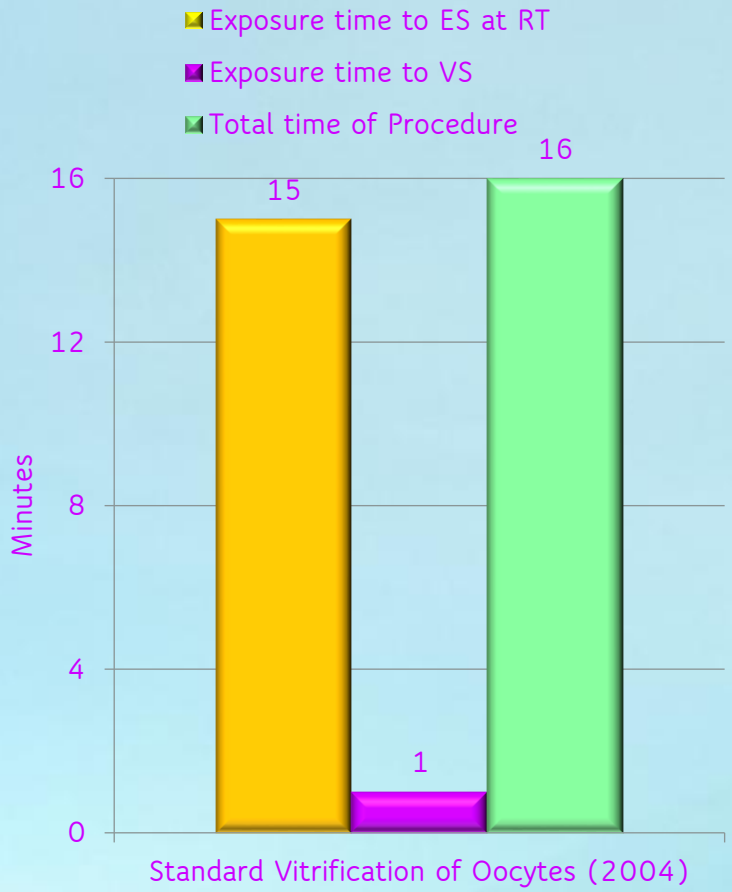
- That means that prolonging the exposure to the CPA solution does not improve the cytosolic glass forming tendency and could be avoided

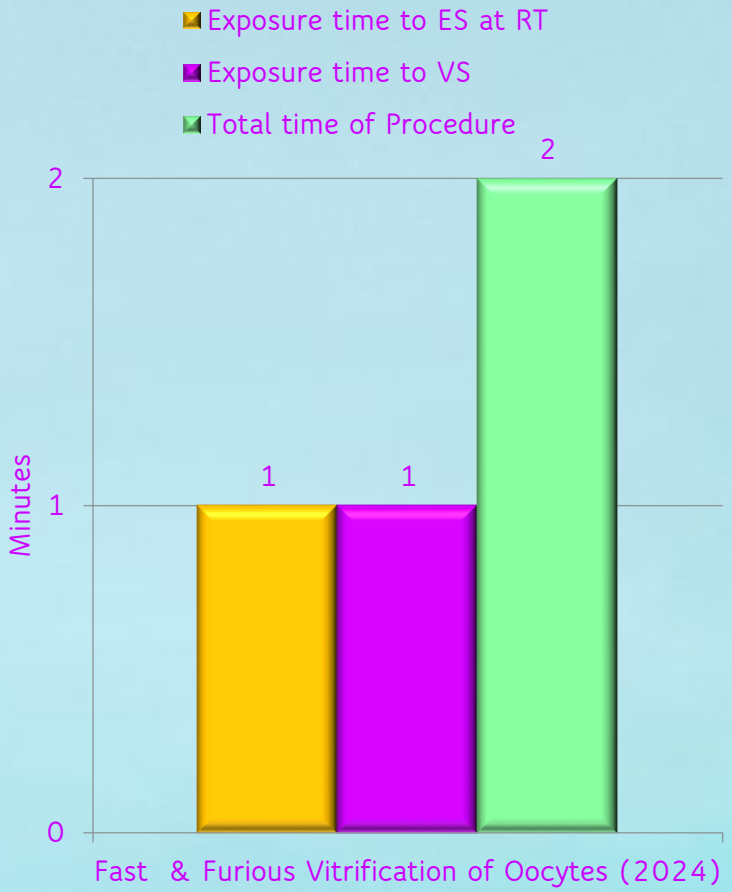


- After 2mins of exposure to standard CPA solutions the critical intracellular solute concentration necessary for successful vitrification was attained

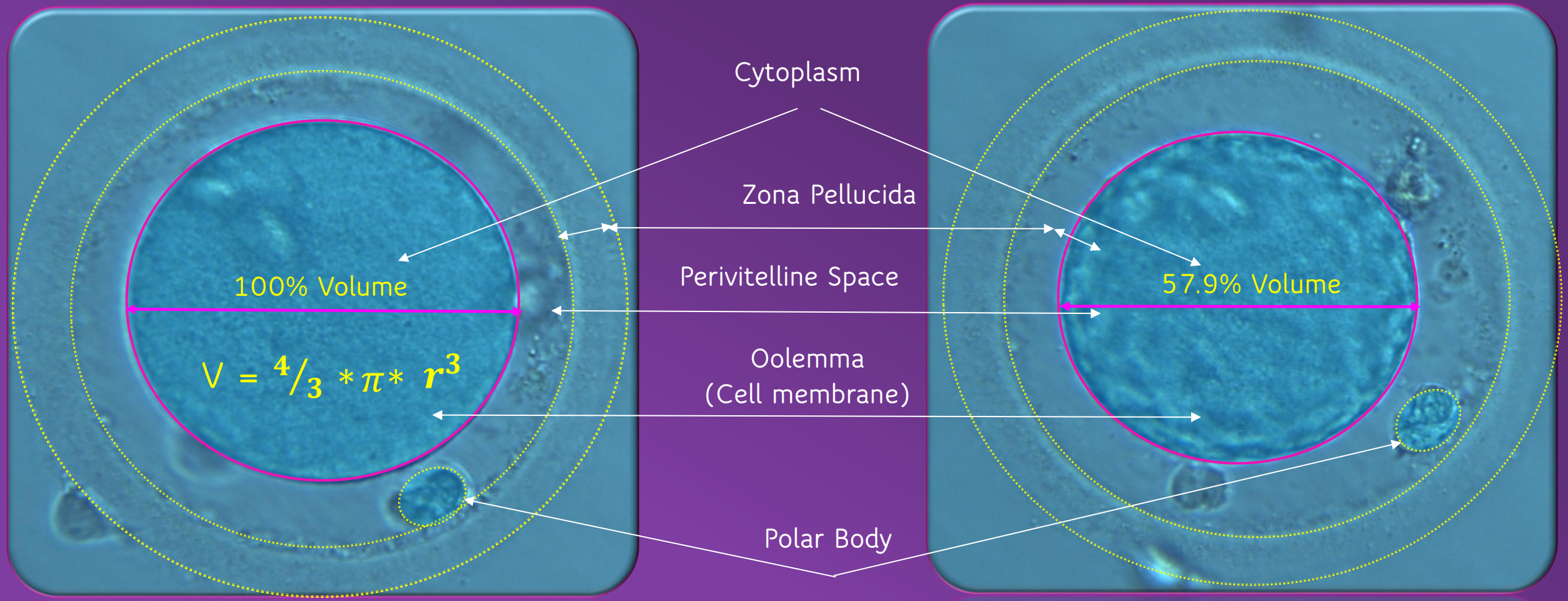
Development of Oocyte Freezing/Vitrification covering 26 years







Volumetric Excursion of a human oocytes before & after 1min exposure to ES (7.5%EG/7.5%DMSO)



100% - 57.9% Volume
Volume Excursion of 42.1%

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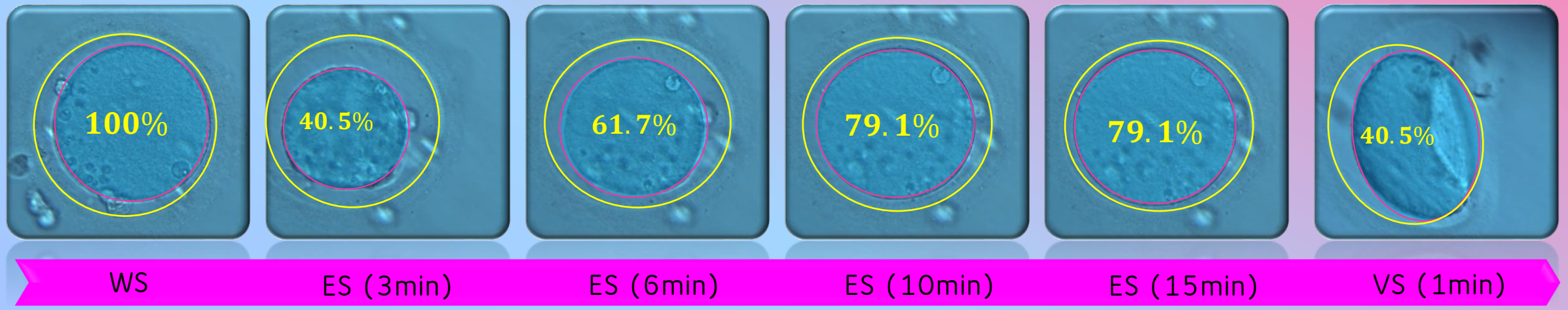
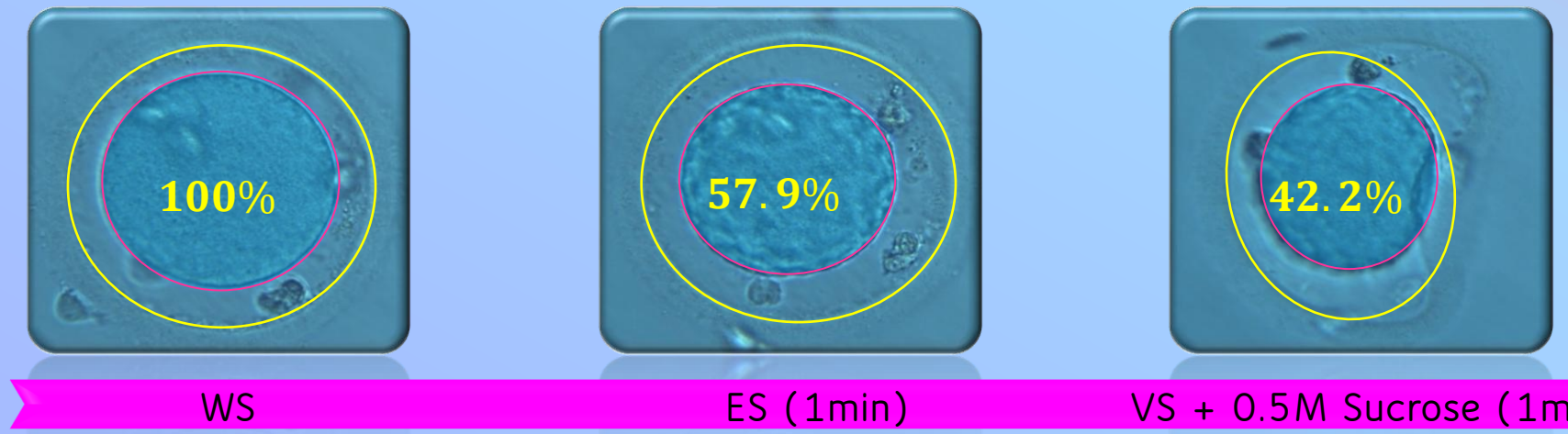
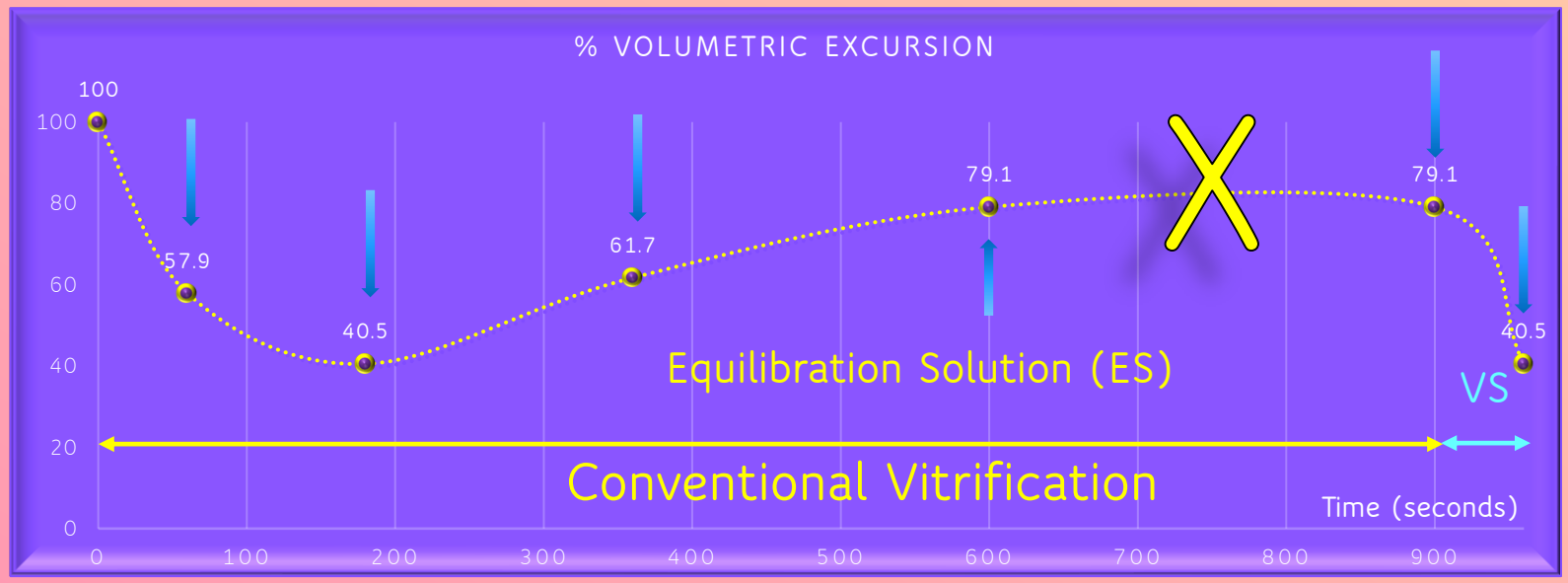
Standard Vitrification – Exposure time to ES 10-15min, then 1min to VS



Ultra-Rapid Vitrification – Exposure time to ES 1min, then 1min to VS

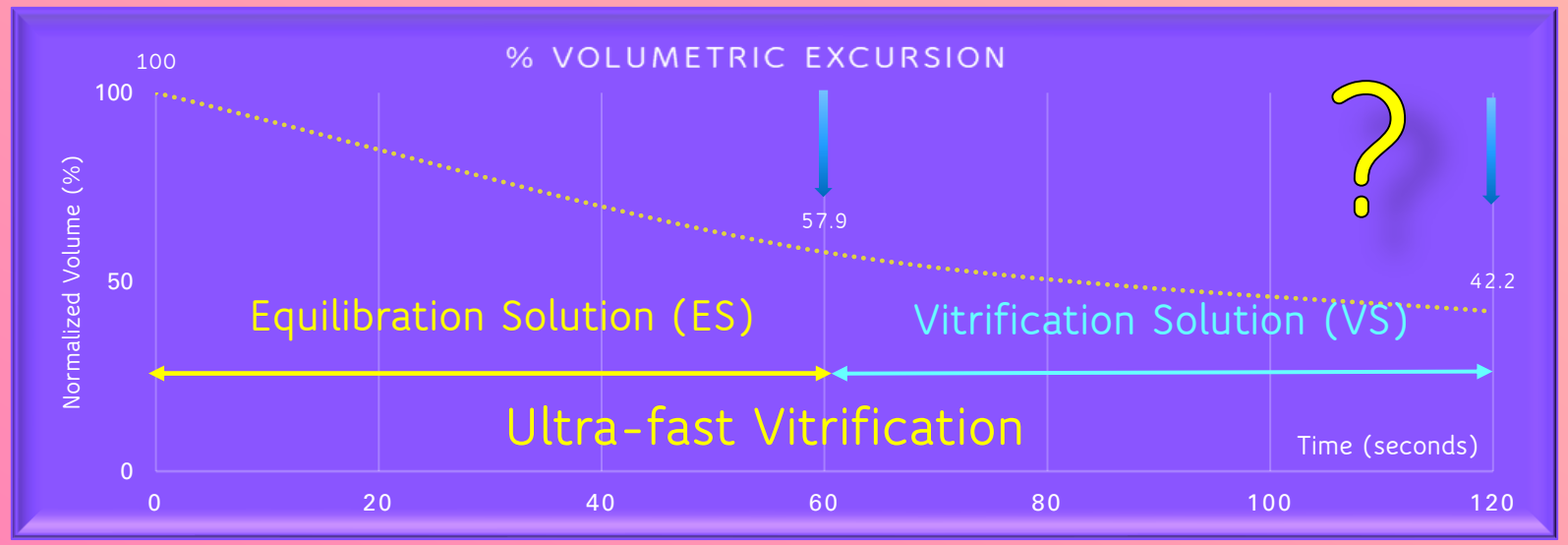




MII Oocytes after dehydration in ES for 3min showing a reduction in volume to 40.5% and after 15min about 80% recovery of the original volume.

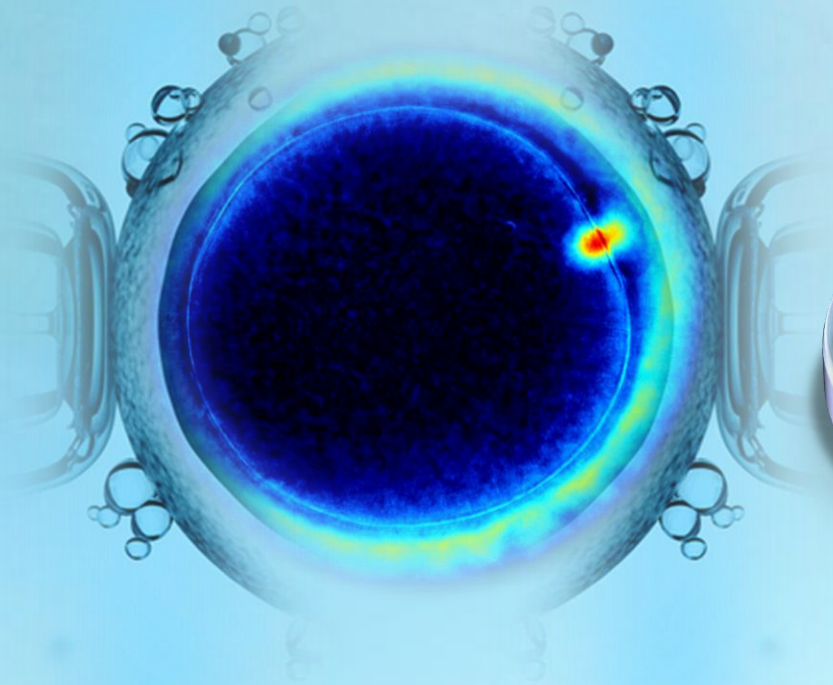
Exposure to VS for 1min reduces the volume again to a final volume excursion of 40.5%

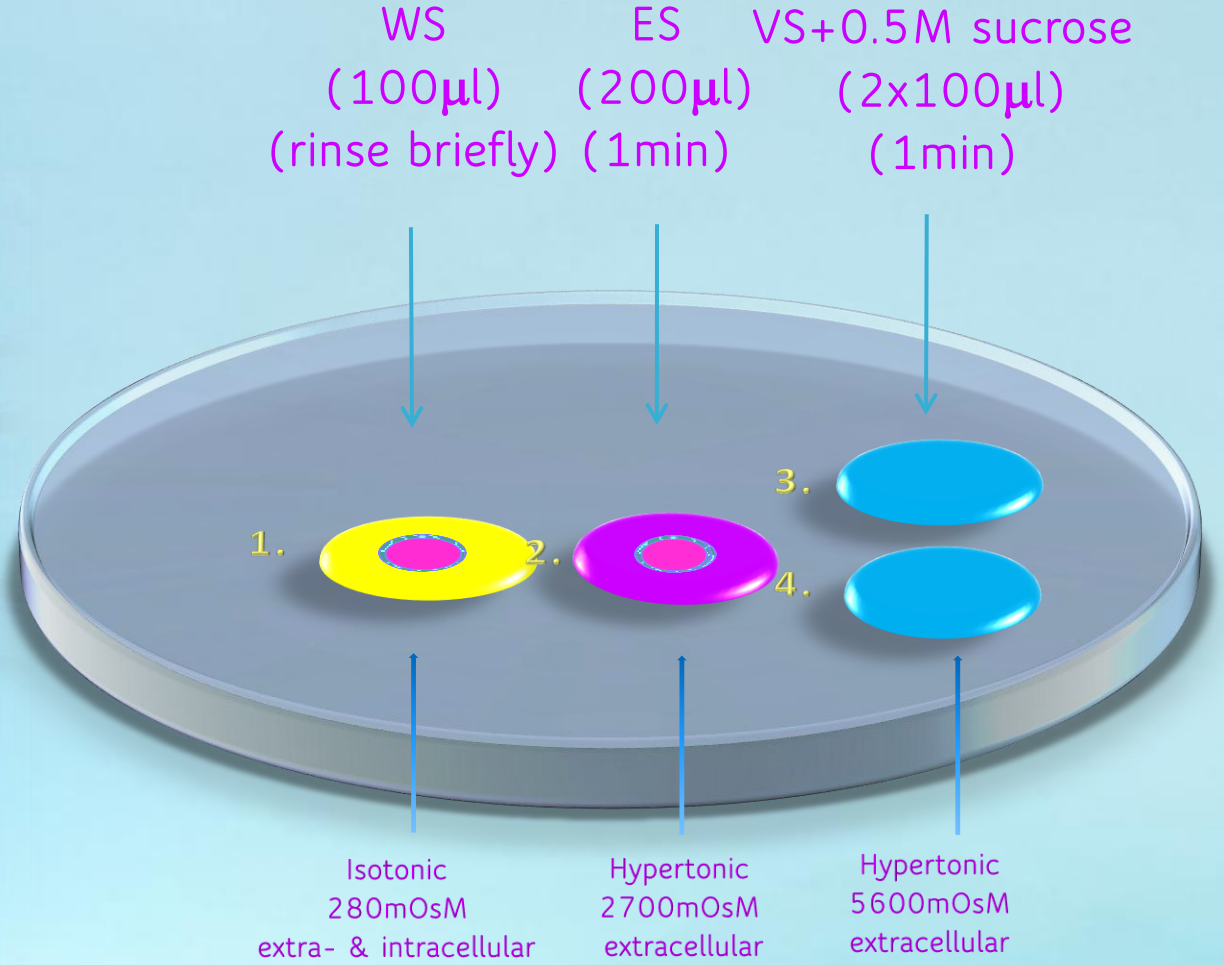
Minimum volume of the shrink curve at about 40%



MII Oocytes after dehydration in ES for 1min showing a volume reduction to 57.9%. Exposure to VS for 1min in VS reduces the volume again to a final volume excursion of 42.2%.

Fast & Furious Oocyte Vitrification Protocol (1 + 1min)





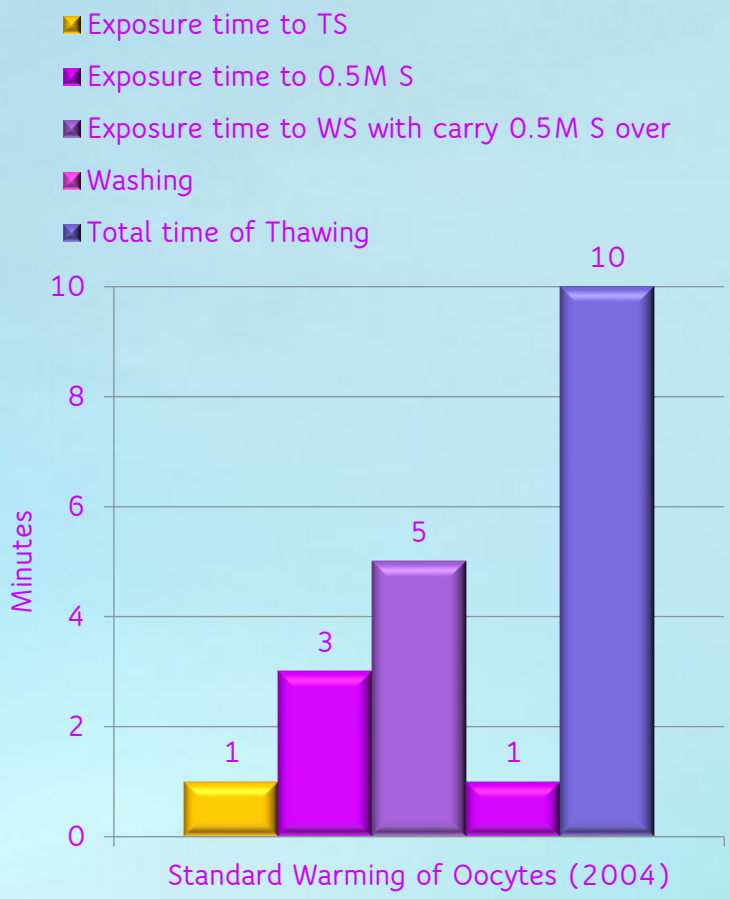
Fast & Furious Oocyte Vitrification Procedure at RT on a plain 90mm Dish Lid Surface (takes 2min)

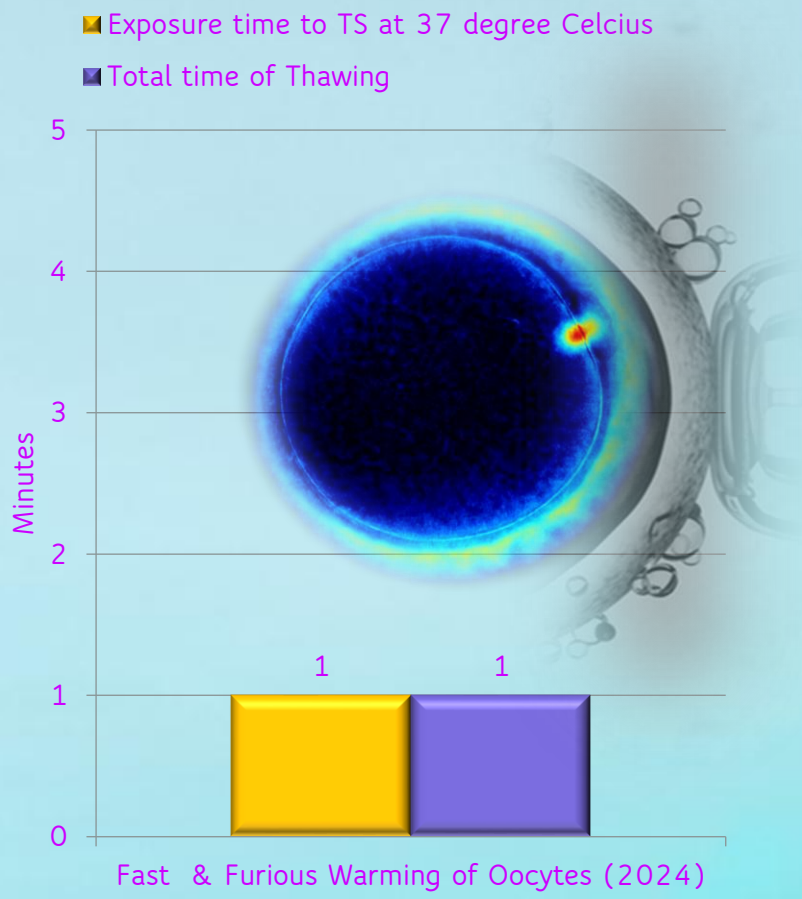
Chang et al., 2022 BOR Vol.106 (2); pp 316-327

Development of Oocyte Thawing/Warming covering 26 years

43% time saving for one Thawing

94% time saving for one Thawing





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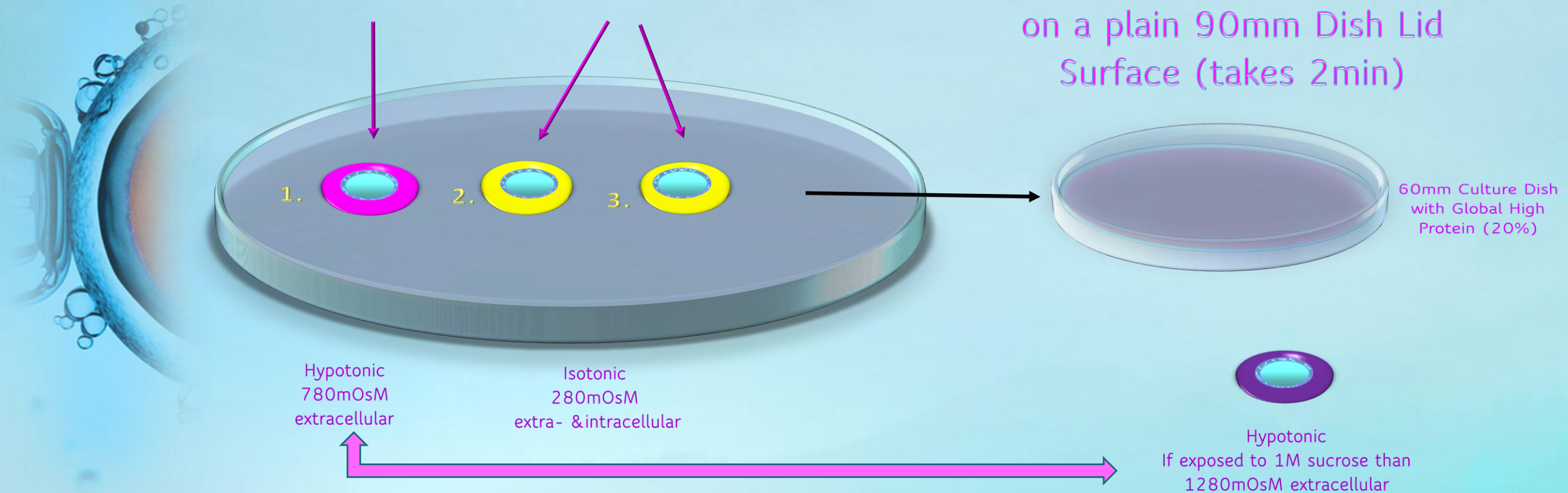
Fast & Furious Oocyte Warming Protocol (1 + 1min) at 37 ° C

0.5M Sucrose: **Δ**mOsM = 780-500= 280 ⟺ 1M Sucrose: **Δ** = 1280-1000 = 280

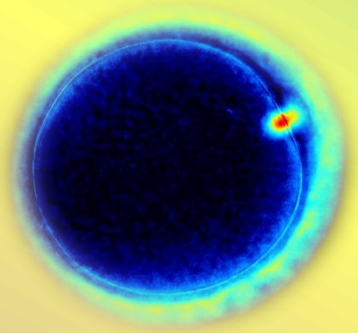
DS
(0.5ml) I (1 min)

WS
(200µl each) I (30sec each)

Fast & Furious Oocyte Warming Procedure at 37°C on a plain 90mm Dish Lid Surface (takes 2min)



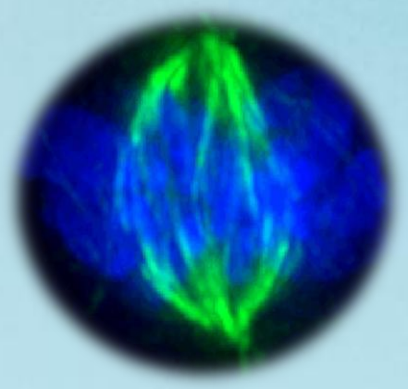
Chang et al., 2022 BOR Vol.106 (2); pp 316-327



Survival of human GV and MI oocytes after vitrification and warming in a total of 4mins in two different media

	Irvine	Vitrolife	χ^2	Irvine	Vitrolife	χ^2
	GV		P-Value	MI		P-Value
n	381	365		156	146	
Survival	360	347	0.77	149	138	0.28
%	94.5	95.0		95.5	94.5	

Resumption of meiotic activity of GV, and MI oocytes and their conversion into MI, and MII 24, and 48 hours post warming[1)] and not vitrified/warmed[2)] in Global HP with 20% Protein



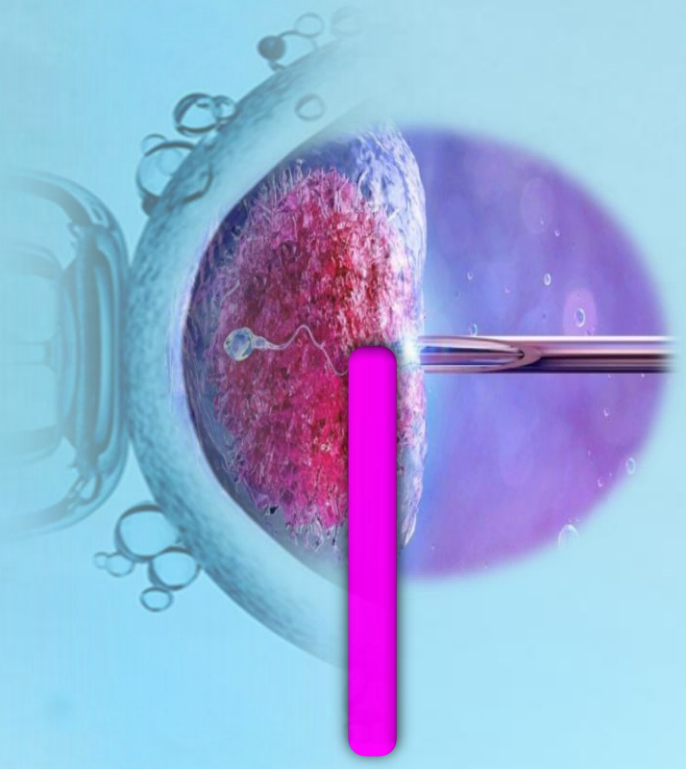
Resumption of meiotic activity of GV, and MI oocytes 24 hours and 48 hours post warming												
	GV[1]	GV[2]		MI[1]	MI[2]	MII[1]	MII[2]	MI[1]	MI[2]		MII[1]	MII[2]
n	746	647						280	539			
Survival	707	NA	Conversion	646	577	424	393	266	NA	Conversion	225	470
%	94.7	NA	%	91.4*	89.2*	60.0	60.7	95.0	NA	%	84.6	87.2
				*P=0.17		*P=0.77					*P=0.31	

[1] Vitrified-warmed; [2] not vitrified-warmed

Survival of unfertilized or converted MII oocytes, abnormal fertilized zygotes, and Cleavage Stage embryos from abnormal fertilized zygotes after 2mins vitrification and 2mins warming in two different media

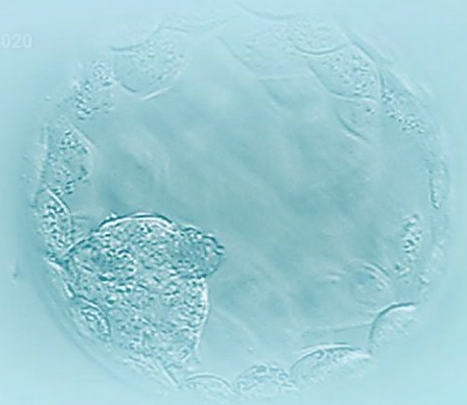
	Irvine	Vitrolife	χ2	Irvine	Vitrolife	χ2	Irvine	Vitrolife	χ2
	MII		P-Value	1 & 3PN		P-Value	Cleavage Stage		P-Value
n	723	614		33	40		56	53	
Survival	661	567	0.50	33	40	ns	55	52	0.96
%	91.4	92.3		100.0	100.0		98.2	98.1	

4 concerning topics:



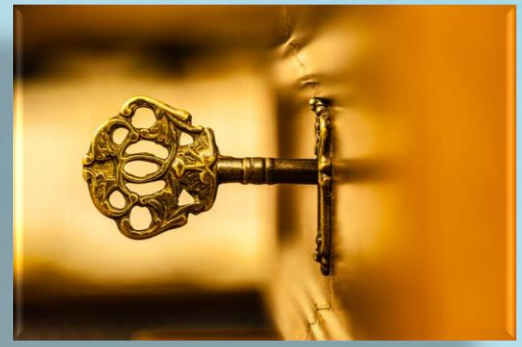
- Interchangeability in terms of cross-over of media types when using different brands
- Number of eggs loaded per device – does it has to be two only?
- Contamination from Liquid Nitrogen

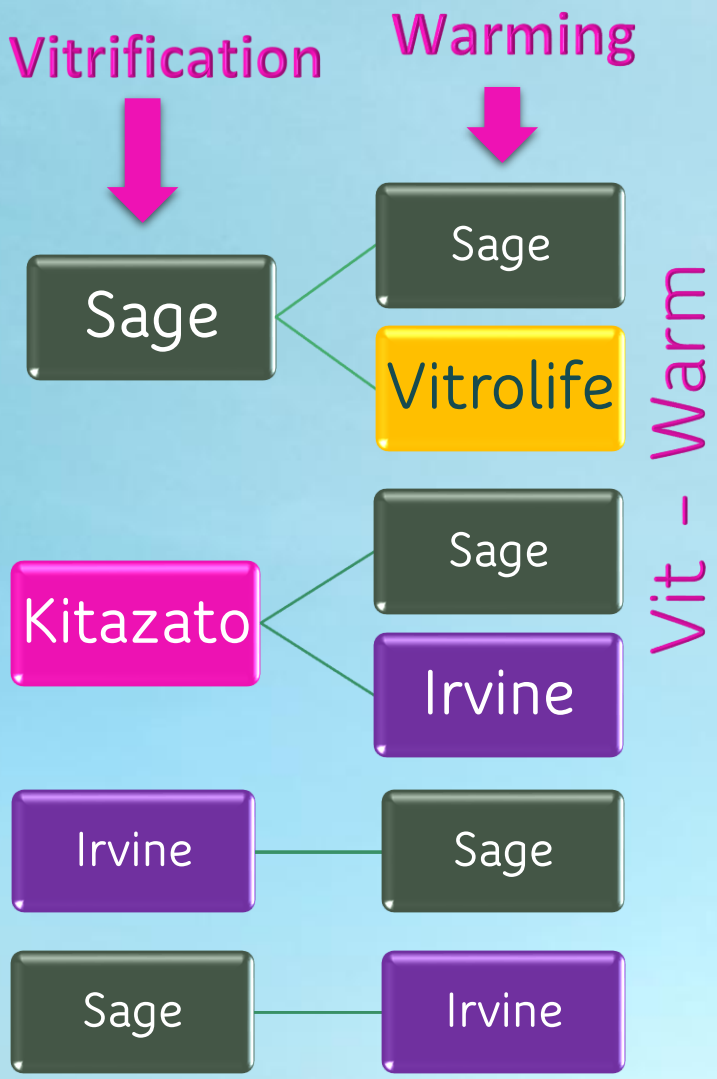
,,

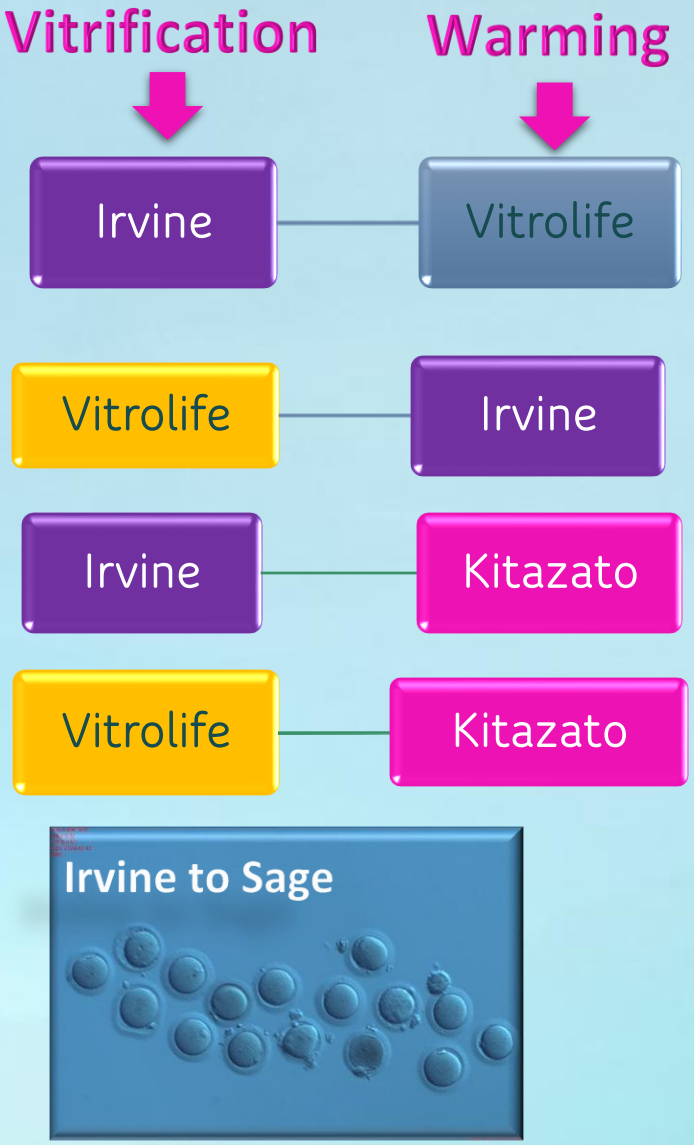


- Universal protocol for eggs, zygotes, and cleavage stage embryo?

Are Media from different Vendors interchangeable?







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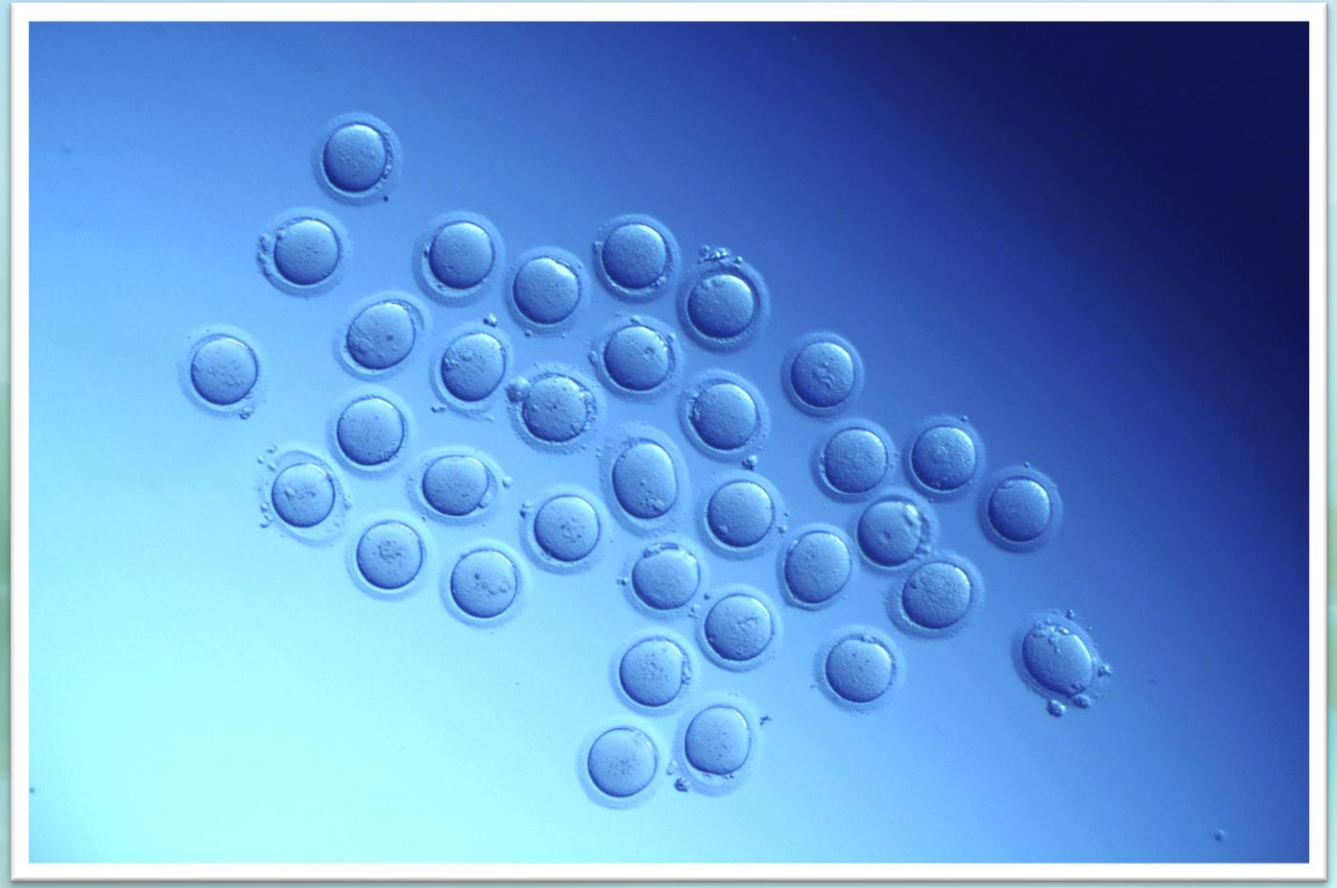
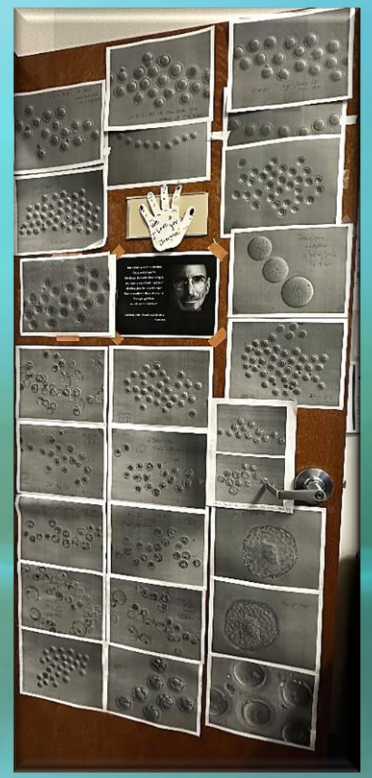
Does it have to be 2 oocytes per carrier only?



Sage: 33xMII vitrified [4x Cryolock].
Survival: 33/33 = 100%

"That's been one of my mantras– focus and simplicity. Simple can be harder than complex: You have to work hard to get your thinking clean to make it simple. But it's worth it in the end because once you get there, you can move mountains."

Steve Jobs



Vitrification

Warming

Simplicity

Efficiency

Vitrification

Warming

Irvine: 16 unfertilized MII vitrified [2x Cryolock with 8] -starting with 16 eggs, all 16 vitrified in 3:35min.

Warming - done with two Cryolock containing a total of 16 eggs - takes a total of 3:16min to warm all 16 eggs.

Survival: 16/16 = 100%

Sage: 7x abnormal fertilized MII vitrified [1x Cryolock] - all 7 vitrified in 2:30min.

Warming - 1xCrylock with 7 - takes a total of 2:23min to warm all 7

Survival; 7/7 = 100%

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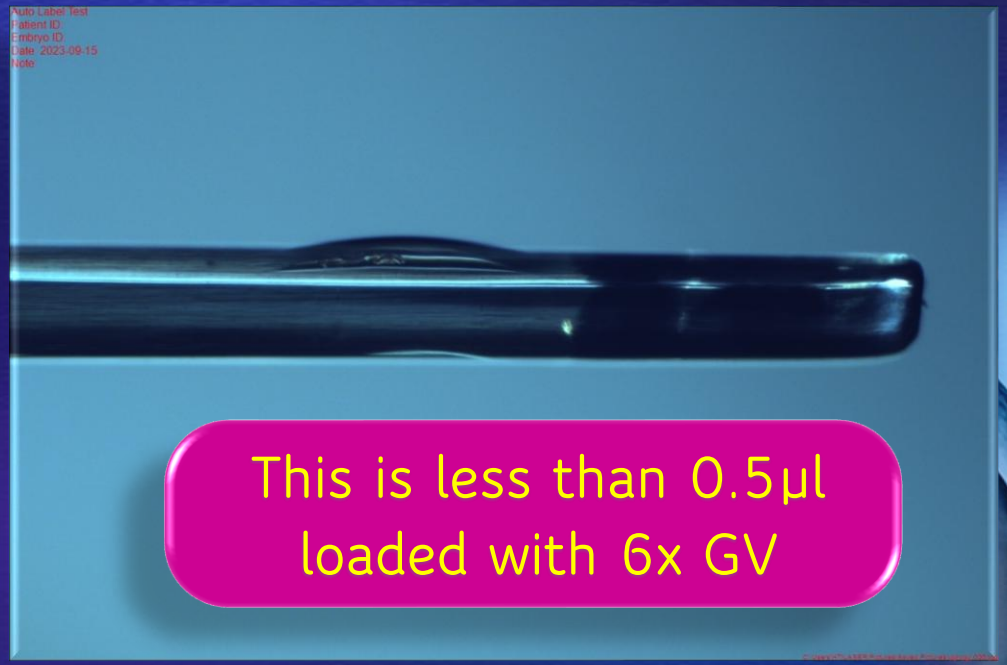
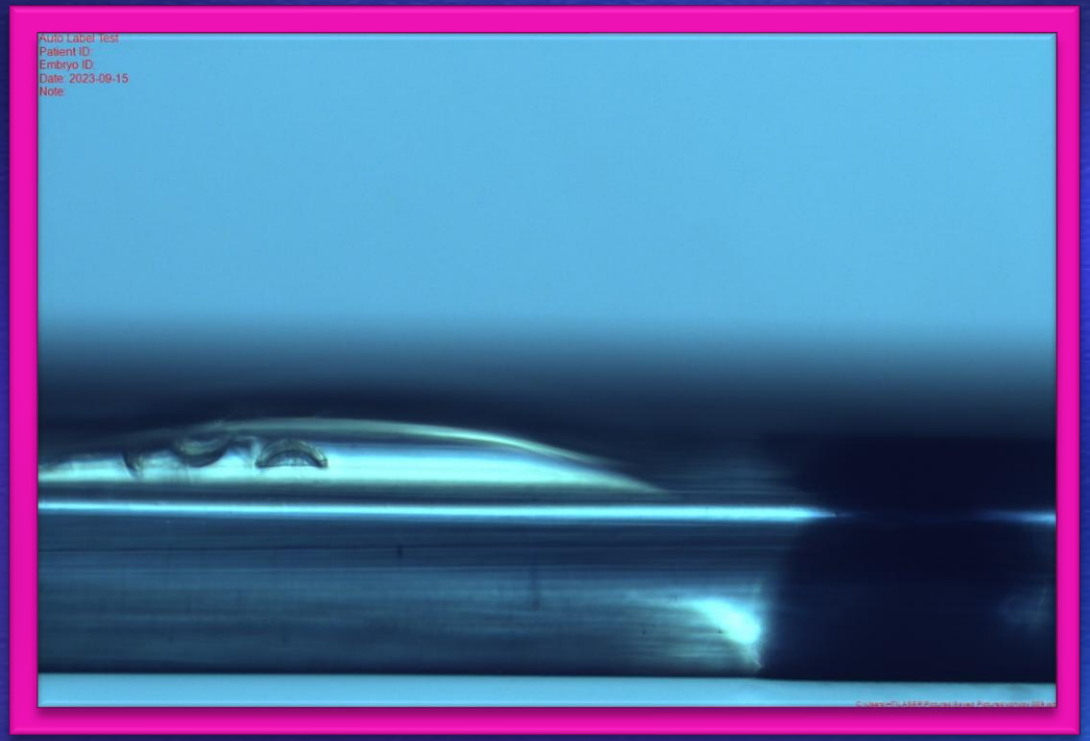
Is volume as important as we all thought?



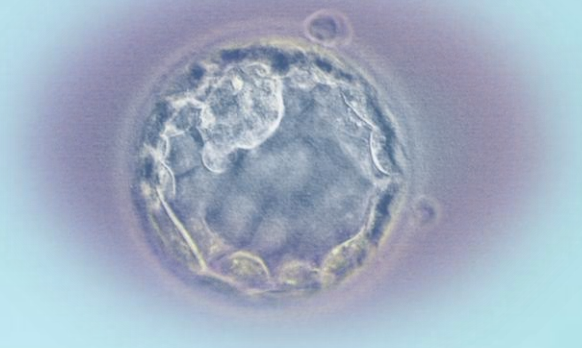


6xGV loaded in less than 0.5µl vitrification solution on a cryolock

“Closed” vs “Open” Systems

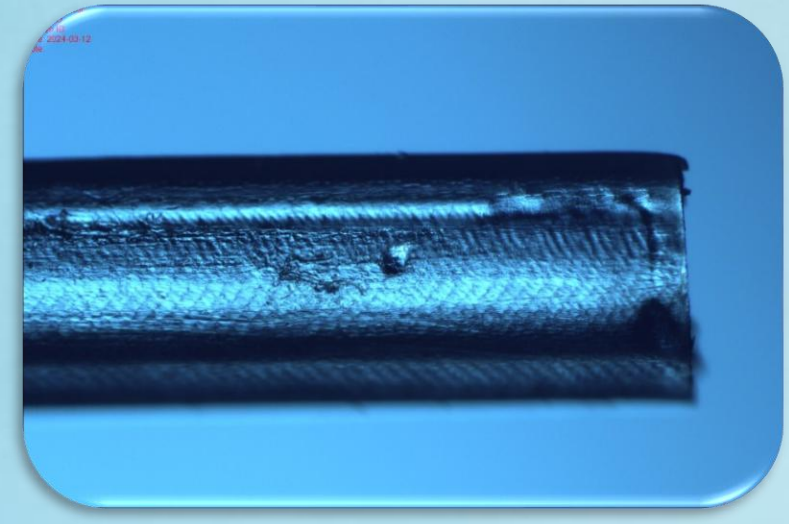
- “Closed” systems (no direct contact with LN2) provide:

  Lower cooling rates (1,500 to 5,000°C/min) because of the reduced heat transfer by the carrier wall



- “Open” systems (direct contact with LN2) provide:

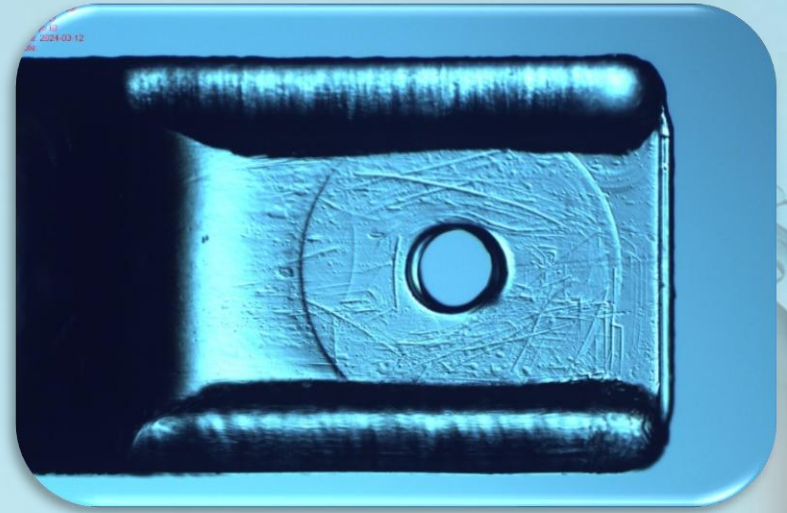
  Higher cooling rates (>20,000°C/min) because of no reduced heat transfer



HSV	N	Survived (n)	Survival (%)
	28	27	96.4

High Security Vitrification kit (HSV)

Irvine Medium used



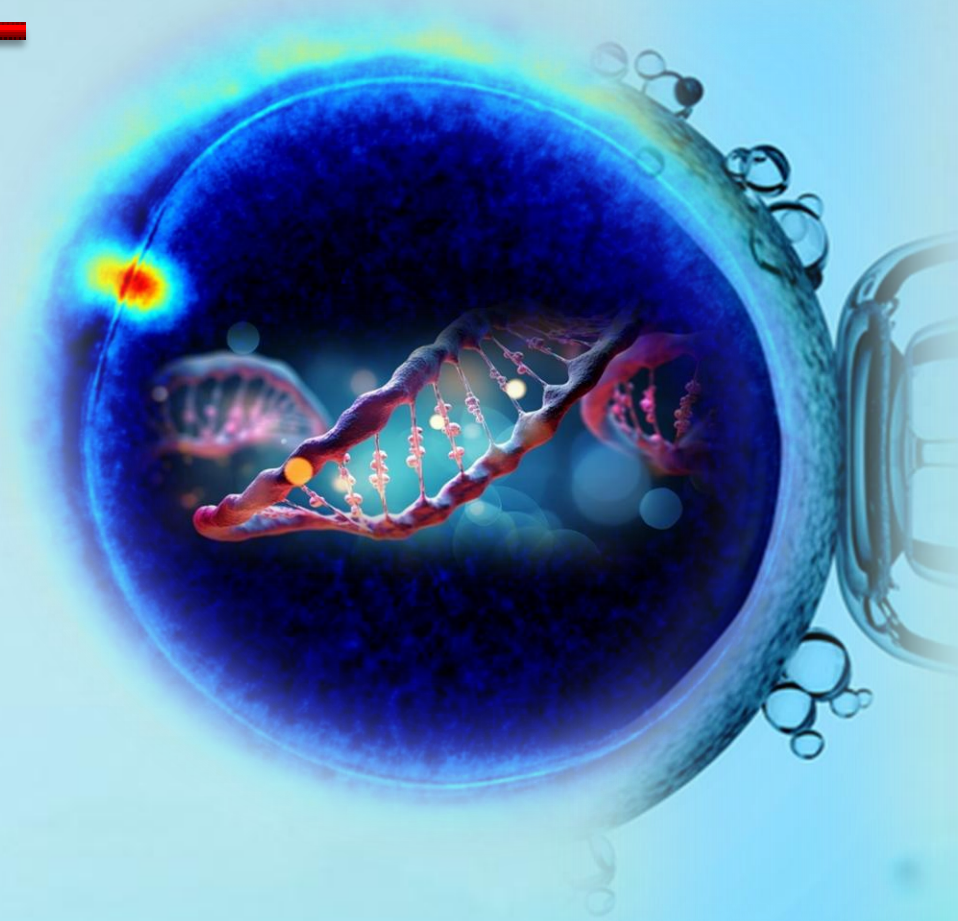
Rapid-I	N	Survived (n)	Survival (%)
	29	29	100

Rapid-I Carrier

Vitrolife Medium used

Ultra-Fast Vitrification: Minimizing the Toxicity of cryoprotective Agents and osmotic Stress in Mouse oocyte Cryopreservation

Jung-Ran Cho et al. Int. J. Mol. Sci. 2024, 25, 1884



Control[1] = Not vitrified

CV[2] = Conventional Vit (10min 7.5% ES; 1min 15% VS)

UFV[3] = Ultrafast Vit (1min 7.5% ES; 1min 15% VS)

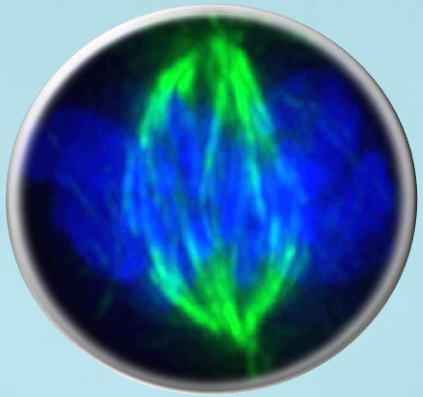
Survival between groups

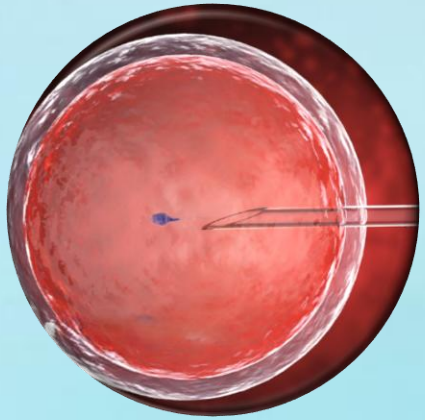
Survival	C[1]	CV[2]	UFV[3]
n	200	210	203
Survival	200	200	200
%	100	95.2	98.5

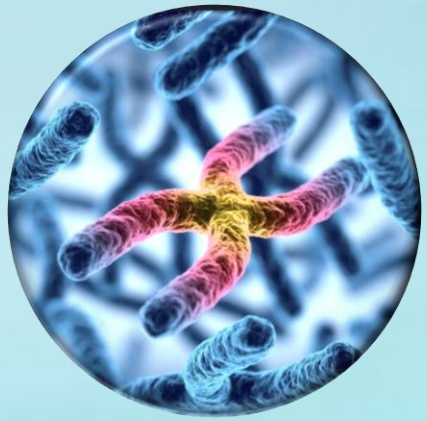
C[1] vs CV[2] [P<0.05]; C[1] vs UFV[3] [P=0.745]; CV[2] vs UFV[3] [P=0.263]

Ultra-Fast Vitrification (UF-VIT) vs conventional slow vitrification (C-VIT) vs Control:

- ✓ Distribution Pattern and Fluorescence Intensity of MT and Mitochondria Membrane Potential (**ΔΨ**m – Key Indicator for Mitochondrial Activity) in favor of UF-VIT
- ✓ Presence of clusters containing Inositol 1,4,5-triphosphate (IP3)* *Inositol plays a structural role in your body as a major component of cell membranes in favor of UF-VIT
- ✓ Morphological appearance of the meiotic spindle (**α**-tubulin) and chromosome - no difference
- ✓ Less relative volume excursion of oocyte over time, initiated by Equilibration solution exposure at t =0 according to UF-V and C-V – in favor of UF-VIT
- ✓ Fertilization, cleavage, and blastocyst formation rates of mouse oocytes following Intracytoplasmic Sperm Injection (ICSI) – in for of UF-VIT







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What about Fertilization, embryo development and outcome?

IVF outcomes

Webinar from
September 2024

	Ultra-rapid vitrification and thawing	Standard vitrification and thawing	P value
Oocytes (n)	531	634	0.0005
Survival	100%	94.5%	0.002
Fertilization rate	89.3%	83.2%	0.011
D3 Development	94,2%	93,7%	0.0005
Blastocyst rate	67.3%	53.0%	0.011
Euploidy rate	61.8%	61.4%	0.0005

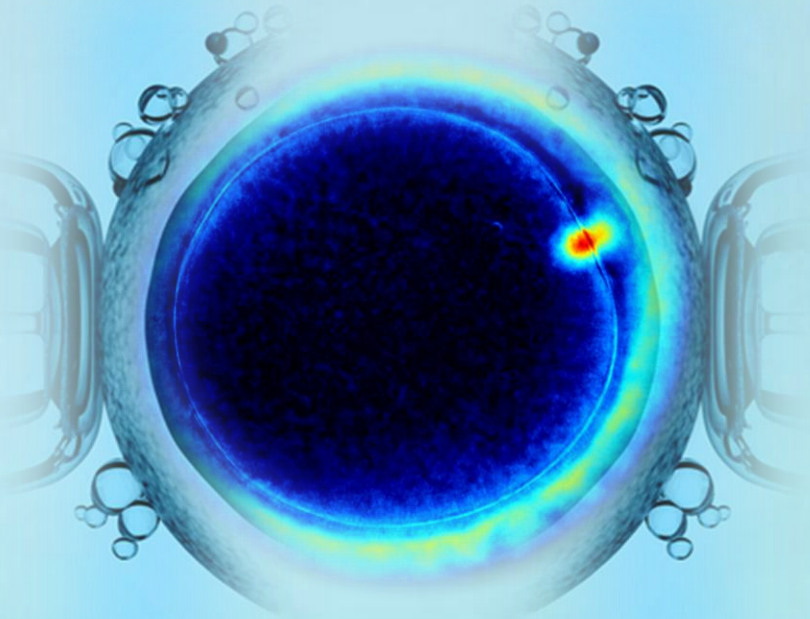
Clinical Pregnancy Rate:
65.2%

Universal Protocol: Combined survival of human oocytes, zygotes & embryos after 2mins vitrification and 2mins warming in different media

Liebermann et al., 2024 RBMOnline Vol. 49 (1):1-3

Irvine, Vitrolife, Sage & Kitazato

	GV	MI	MII	1 & 3PN	Cleavage Stage	Total
n	862	327	1596	86	122	2993
Survival	814	311	1470	86	120	2801
%	94.4	95.1	92.1	100	98.4	93.6



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Vitrification
As with all
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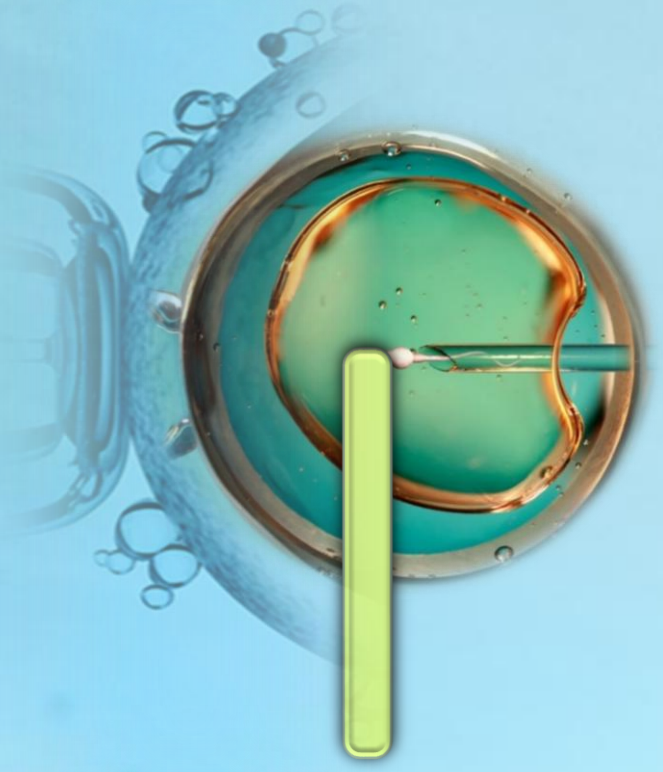
Statement from a presentation I gave on April 9th, 2011, in Valencia at



- Short exposure time during Equilibration (Dehydration)
- Short time for warming (Rehydration)
- Direct Rehydration for Blastocysts (no sucrose exposure)
- Vitrification/warming of oocytes or embryos simplified

Consistently high

1) Survival
2) Ongoing PR
   Implantation
3) Live Births

Future of vitification

Warming Results from a Laboratory/Clinical Perspective vs Fresh elective Day 5 Blastocyst Transfers

PAST – PRESENT - FUTURE

Time frame	2004-2012 Traditional Warming – 9min	10/2022-11/2024 Fast & Furious – 1min	Untested	Euploid	Fresh eSETs on Day 5
N FETs	3439	2070	763	1307	1883
Average age (SEM)	35.2 ±5.1	36.6 ±4.5	35.9 ±4.8	37.0 ±4.3	31.3 ± 3.3
N Blastocysts warmed	6434	2191	856	1335	-
N Blastocysts survived (%)	6334 (97.8)	2187 (99.8)	855 (99.9)	1332 (99.8)	-
N Blastocysts transferred (Mean)	6236 (1.8)	2187 (1.0)	855 (1.1)	1332 (1.0)	1883
N Positive beta (%)	1784 (51.9)	1486 (71.8)	527 (69.1)	959 (73.4)	1322 (70.2)
N Clinical Pregnancy Rate (%)	1533 (44.6)	1278 (61.7)	446 (58.5)	832 (63.7)	1162 (61.7)
N Ongoing Pregnancy Rate (%)	1273 (37.0)	1070 (51.7)	358 (46.9)	712 (54.5)	1049 (55.7)
N Implantation Rate (%)	2007 (32.2)	1321 (60.4)	479 (56.0)	842 (63.2)	1169 (62.1)

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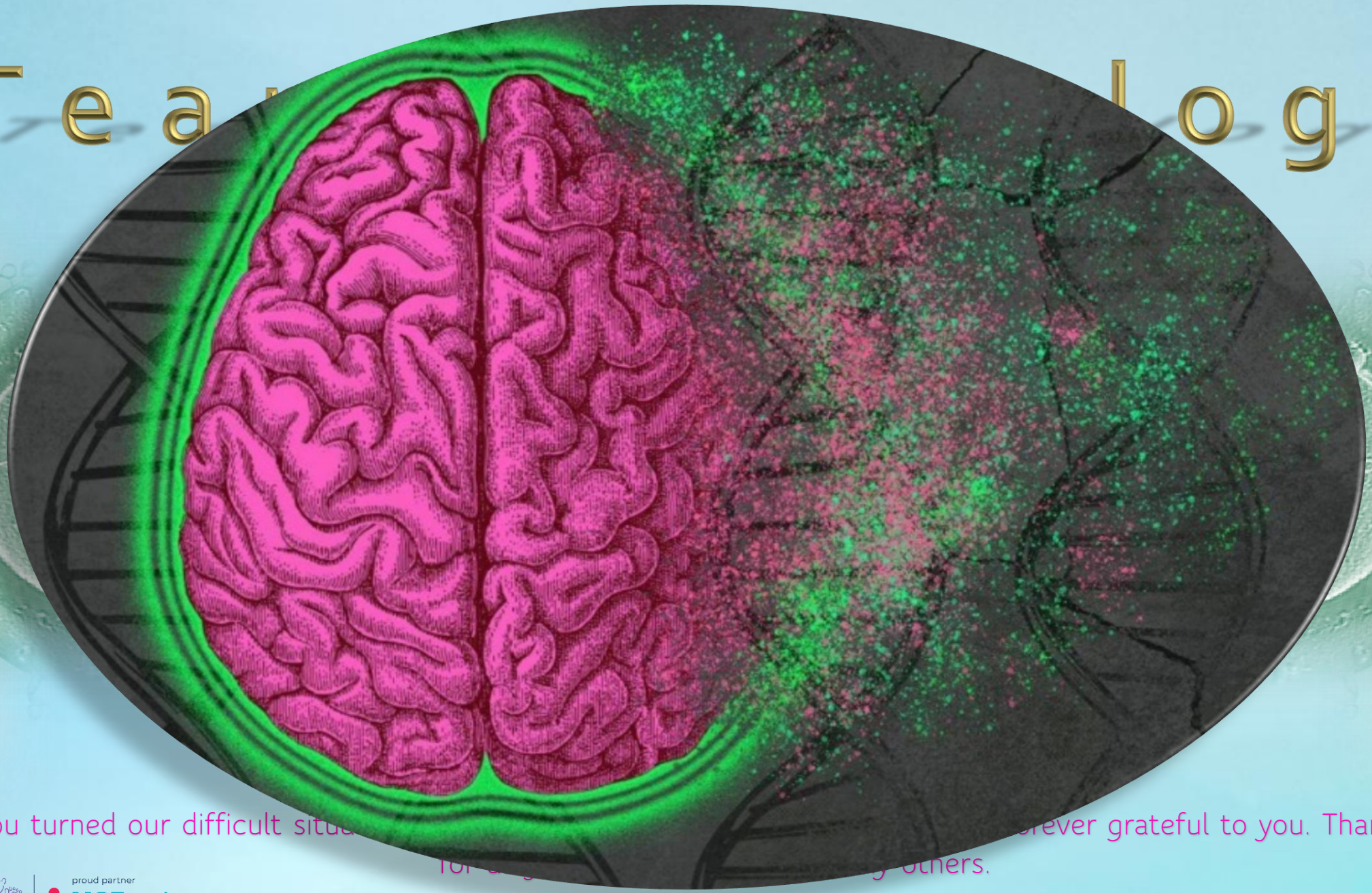

You turned our difficult situ ever grateful to you. Thank you
for g others.

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JOHN F. Kennedy

Change is the law of life. And those who look only to the past or present are certain to miss the future.

In Memoriam

Stanley Leibo

(1937-2014)

William Frederick Rall

(1951-2024)

