Reshaping the Universe of Vitrification

Annual SCARB Meeting

Dana Point, California,

December 6th, 2022

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Director of Laboratory Services | Fertility Centers of Illinois | Chicago, USA

US Fertility Network

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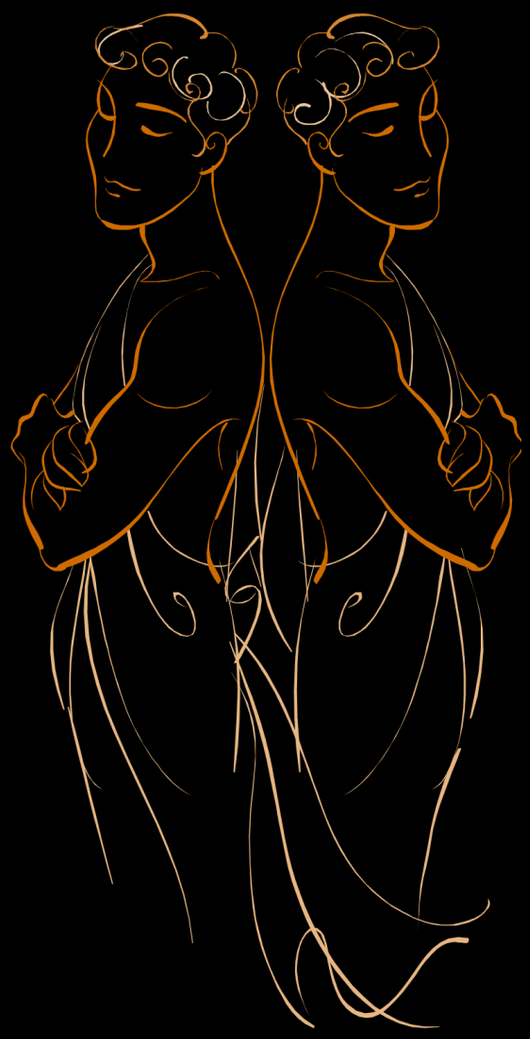

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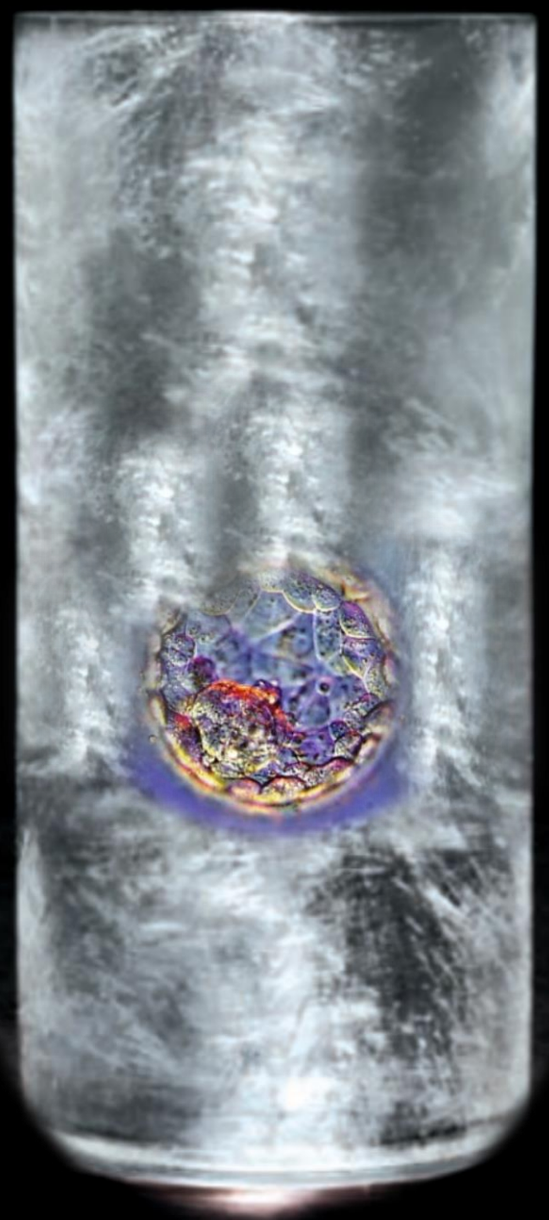

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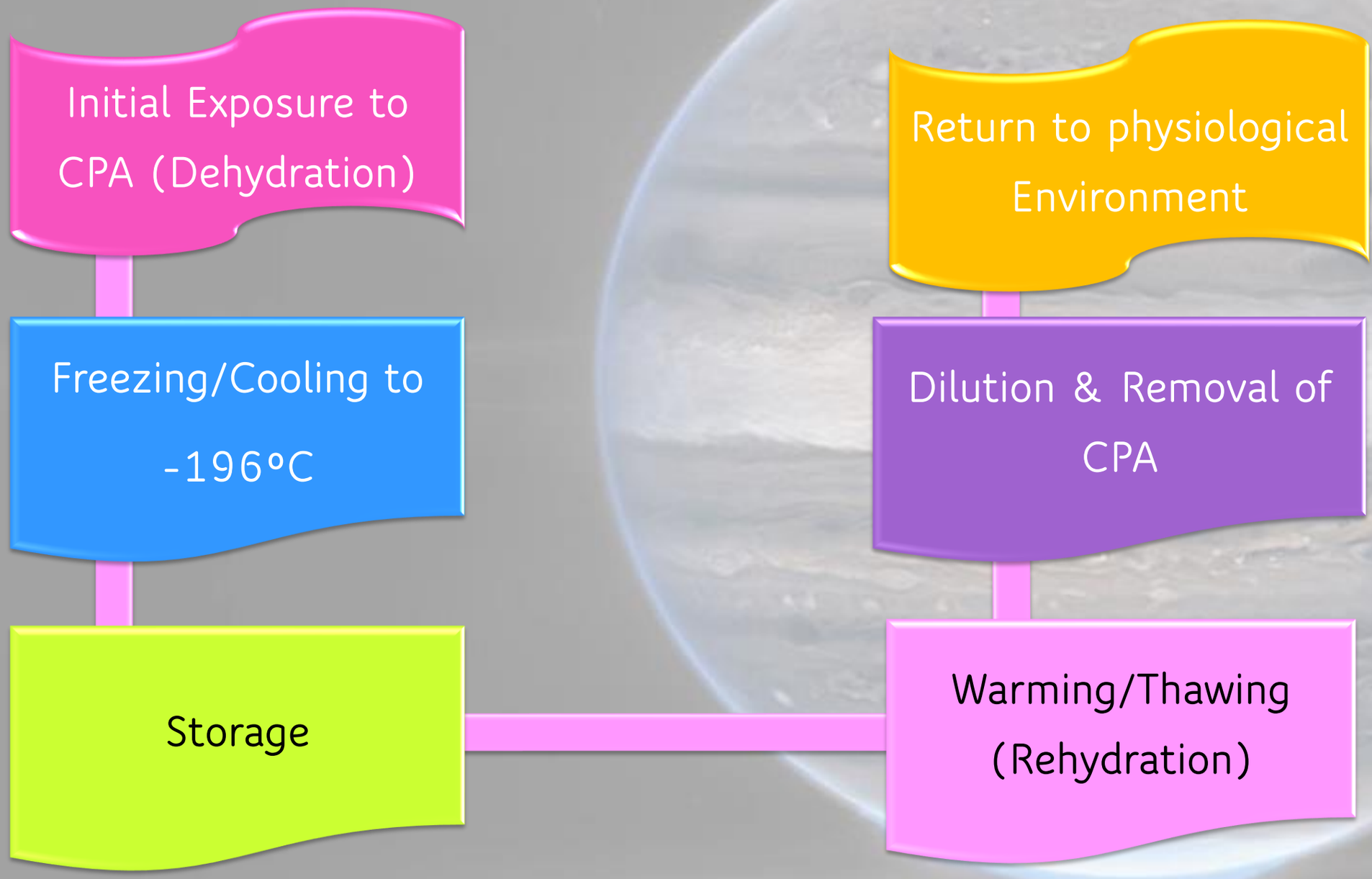
Cryopreservation in General: What Is Involved?



It is simply the ability of being able to prevent ice crystals from forming inside the cell (which can happen during the cooling as well as the warming process)

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CRYO: What are the aims?

Create an intracellular environment that supports the transition from a liquid to a solid glass-like state

The intracellular compartment must be conditioned to allow the emergence and maintenance of a vitreous state

Arrest the metabolism which could then be reversed

Maintain structural & genetic integrity

Achieve acceptable survival rate after thawing

Method must be reliable & repeatable

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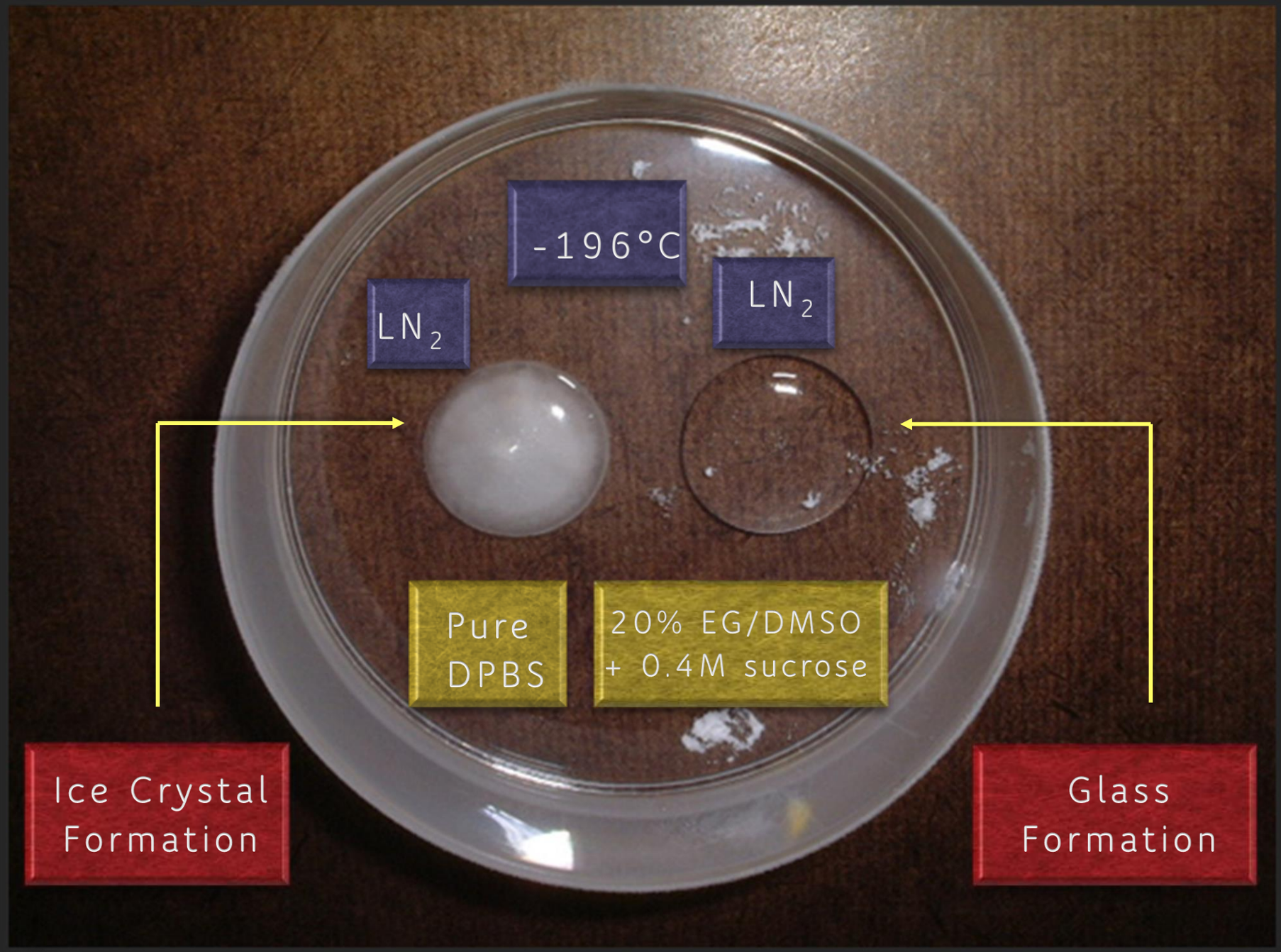

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Physical Definition: Conversion of a superviscous, supercooled liquid into a glassy state when it is cooled below its glass transition temperature (T_g)

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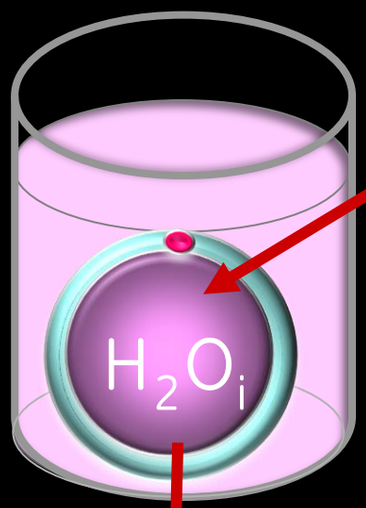
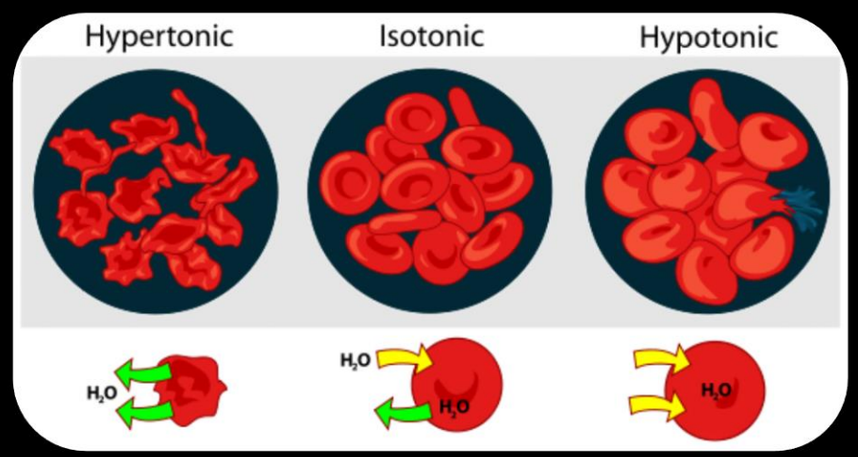
What needs to happen before Vitrification?

Higher extracellular osmolarity

1

EG/DMSO



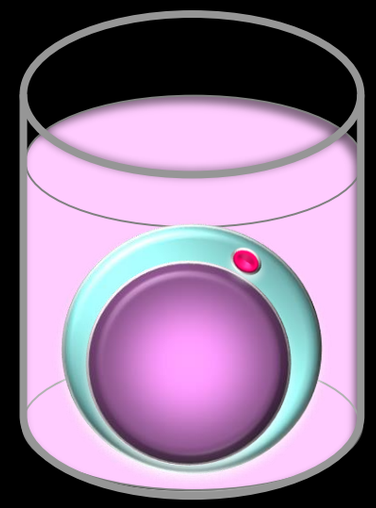


2

Reduction of cell volume caused by dehydration

Equilibrium between intra- & extracellular osmolality

3



Cell turns almost back to the original cell volume

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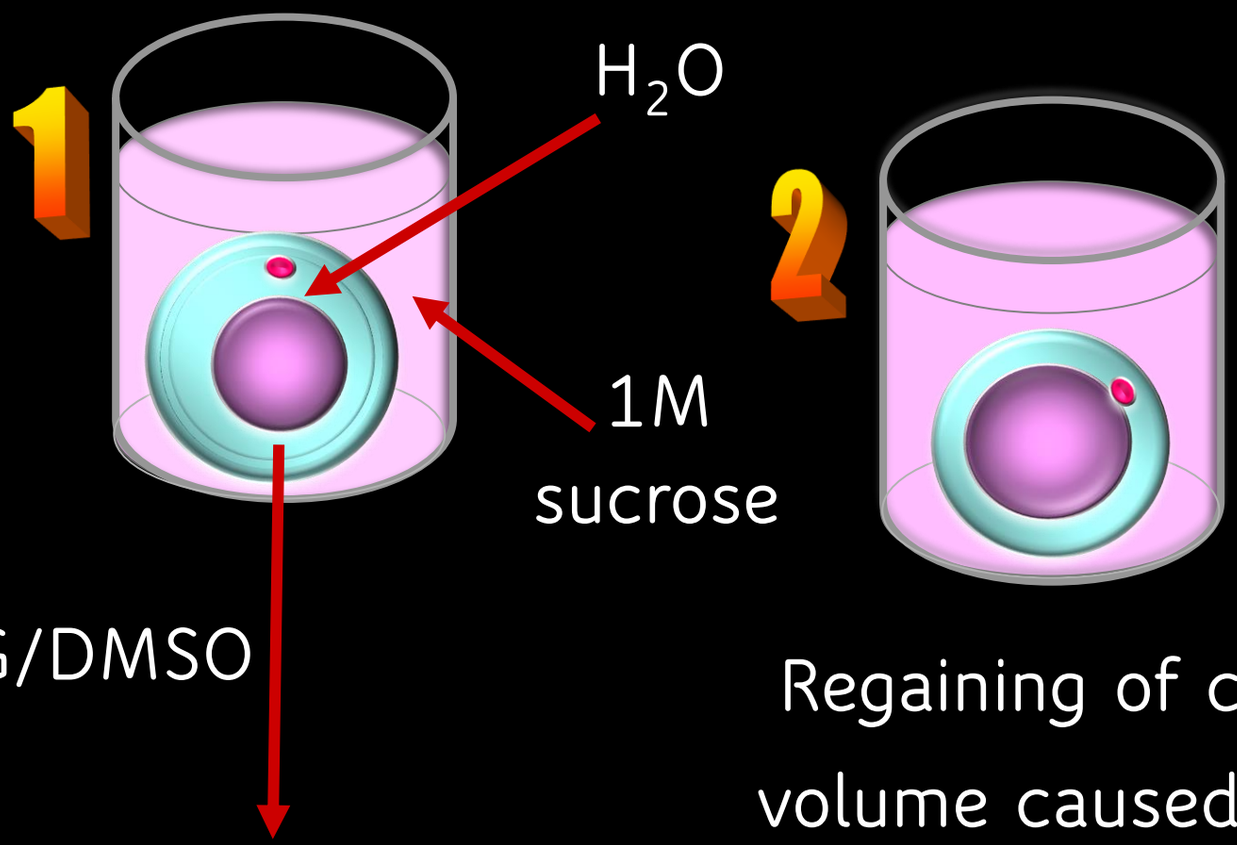
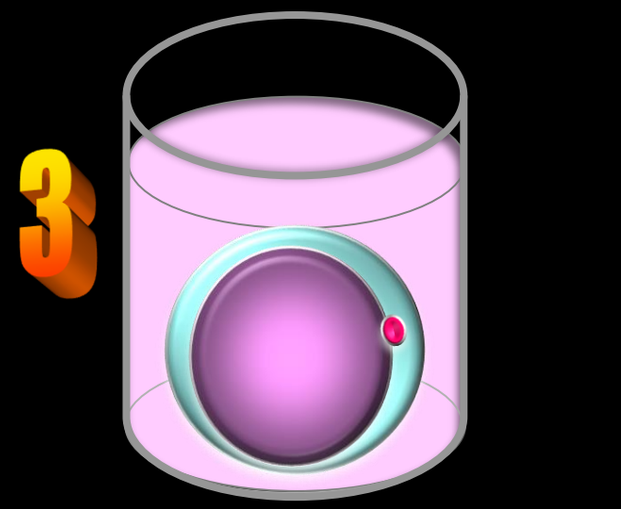
What happens during Warming?

Higher intracellular osmolarity



Regaining of cell volume caused by rehydration

Equilibrium between intra- & extracellular osmolality



Cell turns almost back to the original cell volume

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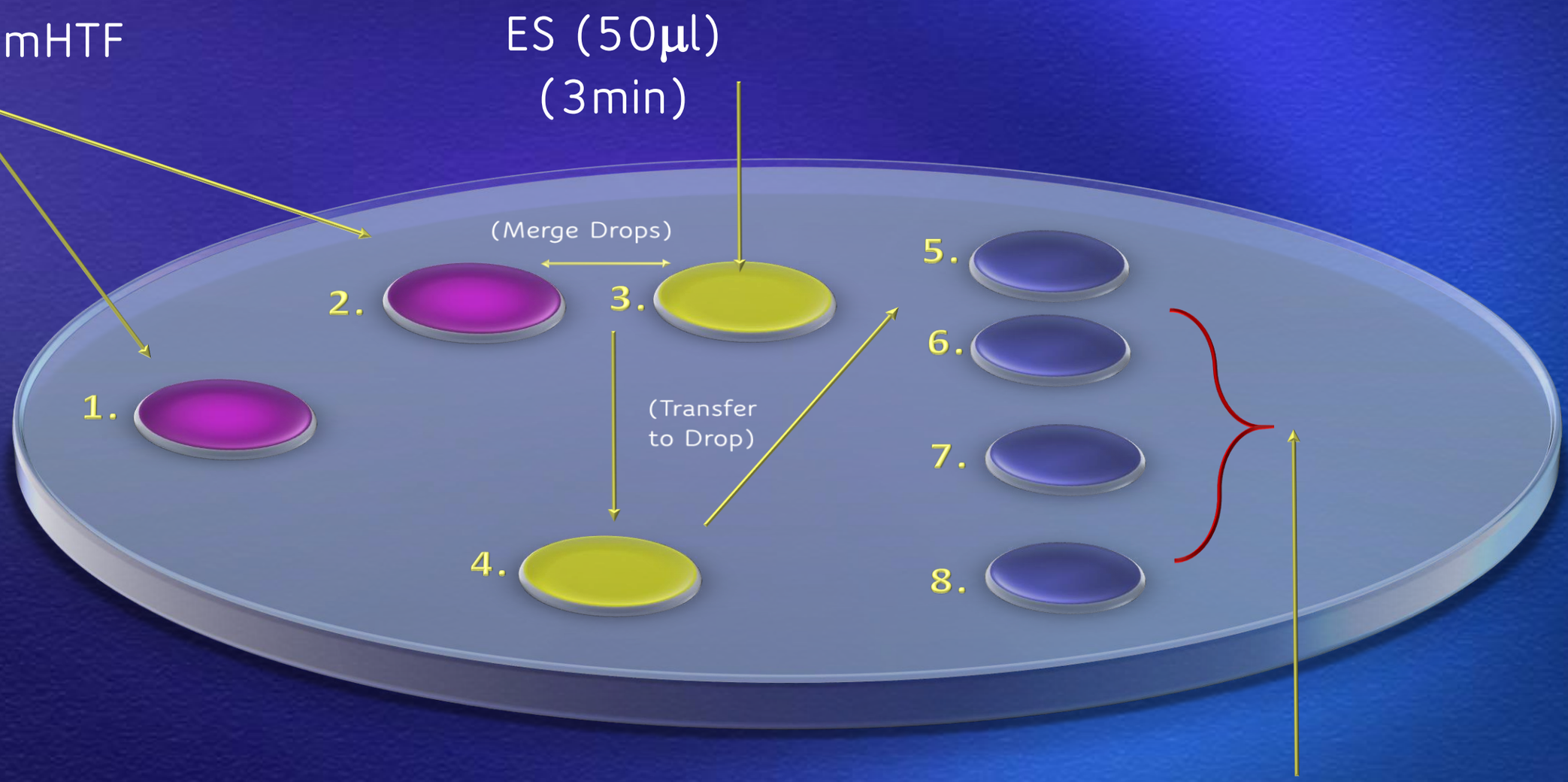
Original Blastozyst Vitrification Procedure on a plain 90mm Dish Lid Surface (takes 9min)



ES (50µl)
(5min)

VS (50µl each)
(1min)

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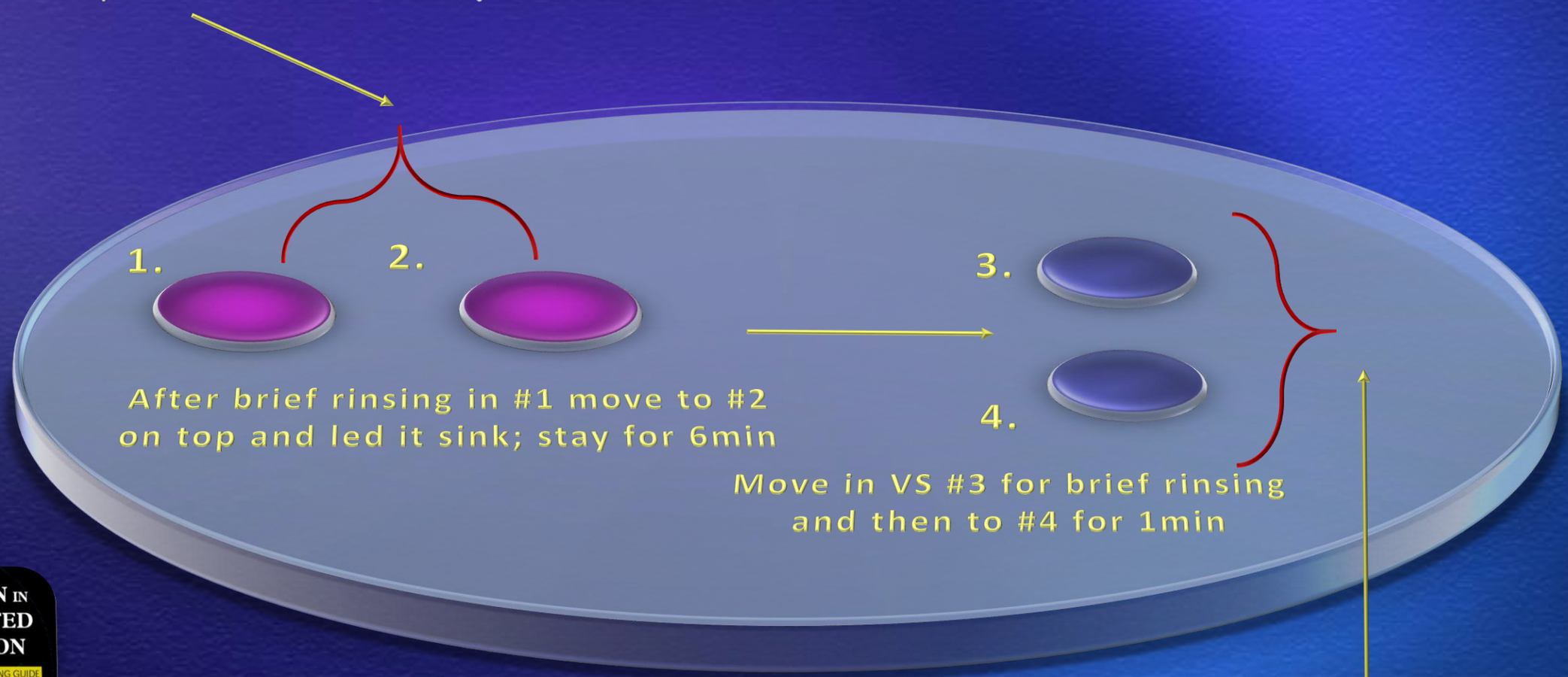
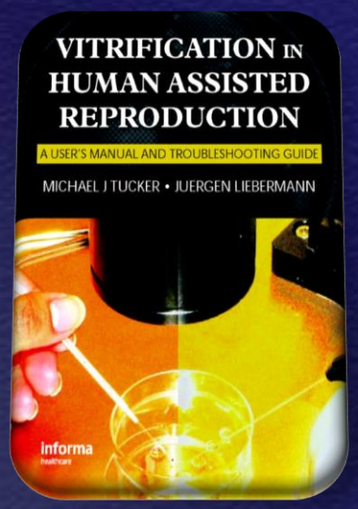
Modified Blastozyst Vitrification Procedure on a plain 90mm Dish Lid Surface (takes 7min)

2x drops of ES (50µl each)





VS (100µl each)
(1min)

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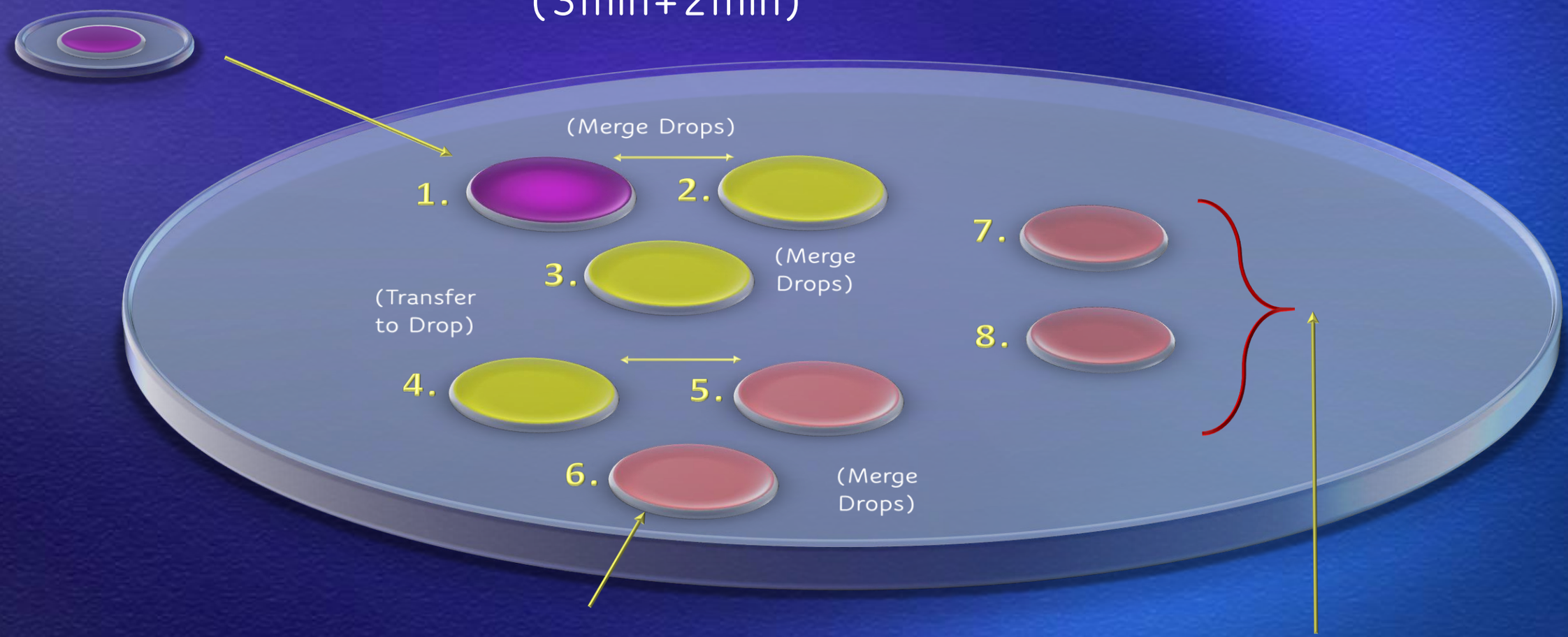
Original Blastozyst Warming Procedure on a plain 90mm Dish Lid Surface (takes 11min)

200µl TS (37°C) on a 90mm Dish Lid surface

TS+DS (50µl) (3min+2min)



DS+WS (50µl) (3min+2min)

VS (50µl each) (1min)

Thawing Solution [TS]

Diluent Solution [DS]

Washing Solution [WS]

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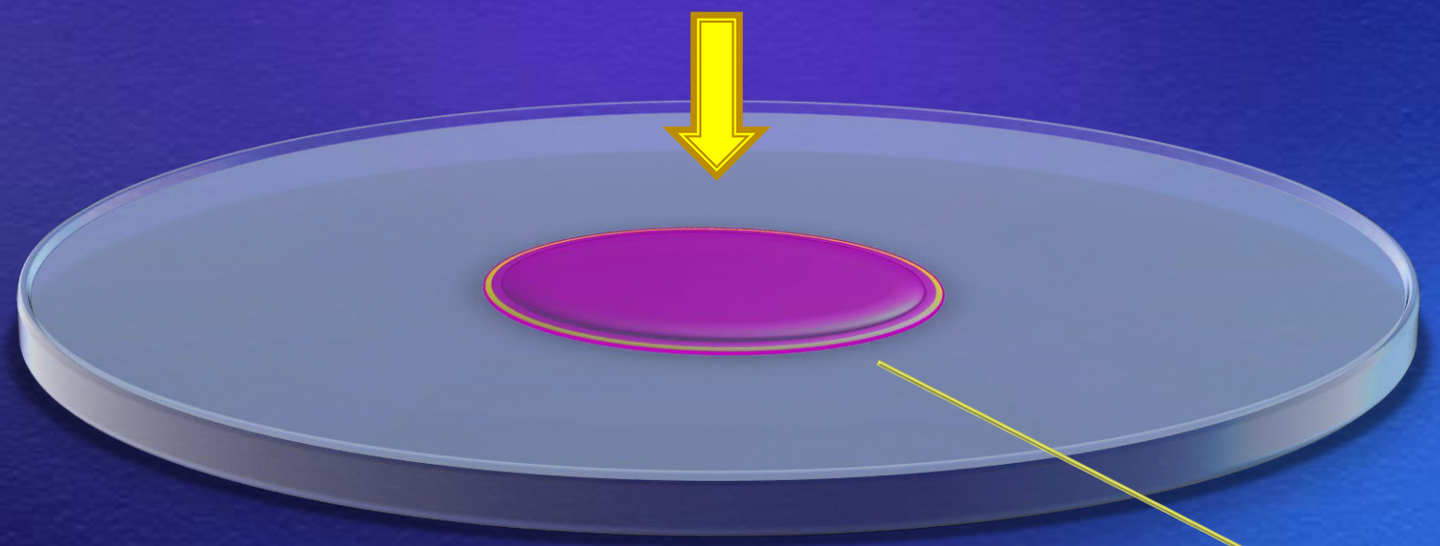
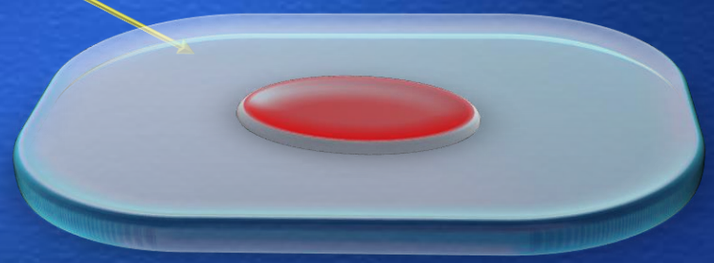
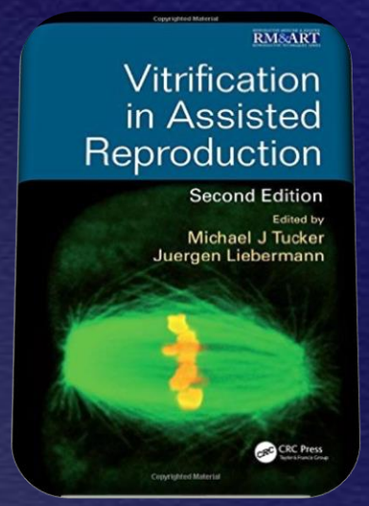
Modified Blastozyst Warming Procedure on a plain 90mm Dish Lid Surface (takes 1min)

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





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Results from a 1min Rapid Warming in TS on donated of research 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
564	545 (96.6%)	518 (91.8%)	394 (69.9%)
N (biopsied)			
336	330 (98.2%)	307 (93.0%)	269 (87.6%)
N (Not biopsied)			
228	215 (94.3%)	211 (92.5%)	125 (59.2%)

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Time-saving per single blastocyst vitrification from a 1min rapid-warming in 1M TS

The ultrafast warming protocol demonstrates similar survival, and outstanding hatching rate 24 hrs post warming. In addition, in a big program like ours with up to 10 FETs per day or 1600 per year, this time-saving protocol is a big win (saving 11 minutes of technician time per warming).

	Traditional	Fast	Traditional	Fast	Traditional	Fast
N warm	**Per warming case**		**Daily**		**Yearly**	
1	11min	1min				
10			110min	10min		
1600					17,600min 293hrs	1600min 26.5hrs

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Positive ß-hCG from 337 Transfers

Age: 36.2±4.7 | Avg Embryos transferred: 1.06)

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Blastocyst Outcome per age after a one-step rapid-warming protocol of 1min in 1M TS

Table 1: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per age after a one-step warming protocol of 1min in 1M TS

	<35	35-37	38-40	>40	Total
n	130	76	69	62	337
pos.	101	60	53	41	255
%	77.7	78.9	76.8	66.1	75.7

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Blastocyst Outcome per age after [A] traditional (three-step - 11 min) and [B] rapid-warming protocol (One-step - 1min in 1M TS)

Table 2: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per age after traditional [A] vs. rapid-warming protocol [B]

	<35 A	<35 B	35-37 A	35-37 B	38-40 A	38-40 B	>40 A	>40 B	Total A	Total B
n	364	130	285	76	231	69	150	62	1030	337
pos.	282	102	201	60	156	53	94	41	733	255
%	77.3	77.7	70.7	78.9	67.5	76.8	62.0	66.1	71.2	75.7

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Blastocyst Outcome per day of development after a one-step rapid-warming protocol of 1min in 1M TS

Table 3: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per day of development after a one-step rapid-warming protocol of 1min in 1M TS				
	Day 5	Day 6	Day 7	Total
n	170	160	7	337
pos.	133	116	6	255
%	78.2	72.5	83.3	75.7

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Blastocyst Outcome per day of development after [A] traditional (three-step - 11 min) and [B] rapid-warming protocol (one-step - 1min in 1M TS)

Table 4: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per age after traditional [A] vs. rapid-warming protocol [B]								
	Day 5		Day 6		Day 7		Total	
	A	B	A	B	A	B	A	B
n	579	170	441	160	10	7	1030	337
pos.	427	133	300	116	6	6	733	255
%	73.7	78.2	68.0	72.5	60	85.7	71.2	75.7

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Blastocysts Outcome of untested vs. euploid after a one-step rapid-warming protocol of 1min in 1M TS

Table 5: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) of untested vs euploid after a one-step warming protocol of 1min in 1M TS			
	Untested	Euploid	Total
n	124	213	337
pos.	94	161	255
%	75.8	75.6	75.7

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Blastocysts Outcome of untested vs. euploid [A] traditional (three-step - 11 min) and [B] rapid-warming protocol (one-step - 1min in 1M TS)

Table 6: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per age after traditional [A] vs. rapid-warming protocol [B]						
	Untested		Euploid		Total	
	A	B	A	B	A	B
n	433	124	597	213	1030	337
pos.	312	94	421	161	733	255
%	72.1	75.8	70.5	75.6	71.2	75.7

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Blastocyst Outcome per day of development and tested vs euploid after a one-step rapid-warming protocol of 1min in 1M TS

Table 7: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per age after a one-step warming protocol of 1min in 1M TS

	Day 5	Day 5	Day 5	Day 6	Day 6	Day 6	Day 7	Day 7	Day 7	Total
	Total	untested	Euploid	Total	untested	euploid	Total	untested	euploid	
n	170	70	100	160	52	108	7	2	5	337
pos.	133	53	80	116	38	78	6	2	4	255
%	78.2	75.7	80.0	72.5	73.1	72.2	85.7	100.0	80.0	75.7

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Blastocyst Outcome per age and untested vs euploid after a one-step rapid-warming protocol of 1min in 1M TS

Table 8: Day 5/6/7 Blastocysts Outcome (positive ß-hCG) per age and untested vs euploid after a one-step warming protocol of 1min in 1M TS

	<35 [no PGT]	<35 [PGT]	35-37 [no PGT]	35-37 [PGT]	38-40 [no PGT]	38-40 [PGT]	>40 [no PGT]	>40 [PGT]	Total
n	59	71	28	48	15	53	24	39	337
pos.	47	54	23	37	11	41	14	28	255
%	79.7	76.1	82.1	77.1	73.3	77.4	58.3	71.8	75.7

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Are ongoing pregnancy rates & implantation rates holding

up to the initial high positive ß-hCG's?

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Blastocyst Outcome per age after a one-step rapid-warming protocol of 1min in 1M TS

Table 1B: Day 5/6/7 Blastocysts Outcome [pos. ß-hCG, cPR, oPR, IR] per age after a one-step warming protocol of 1min in 1M TS					
	<35	35-37	38-40	>40	Total
n	83	52	48	44	227
pos.	68	41	34	28	171
%	81.9	78.8	70.8	63.6	75.3
cPR	64	38	31	21	154
%	77.1	73.1	64.6	47.7	67.8
oPR	63	38	29	20	150
%	75.9	73.1	60.4	45.5	66.1
# E. Transf.	90	53	53	50	246
# Implant.	69	38	33	22	162
% IR	76.7	71.7	62.3	44.0	65.9

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Blastocyst Outcome per day of development after a one-step rapid-warming protocol of 1min in 1M TS

Table 2B: Day 5/6/7 Blastocysts Outcome [pos. ß-hCG, cPR, oPR, IR] per day of development after a one-step rapid-warming protocol of 1min in 1M TS				
	Day 5	Day 6	Day 7	Total
n	114	108	5	227
pos.	88	79	6	171
%	77.2	73.1	80.0	75.3
cPR	84	67	3	154
%	73.7	62.0	60.0	67.8
oPR	82	65	3	150
%	71.9	60.2	60.0	66.1
# E. transf.	121	118	7	246
#	88	71	3	162
% IR	72.7	60.2	42.9	65.9

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Blastocyst Outcome per untested vs. euploid blastocysts after a one-step rapid-warming protocol of 1min in 1M TS

Table 3: Day 5/6/7 Blastocysts Outcome [pos. ß-hCG, cPR, oPR, IR] of untested vs euploid after a one-step warming protocol of 1min in 1M TS

	Untested	Euploid	Total
n	86	141	227
pos.	63	108	171
%	73.3	76.6	75.3
cPR	56	98	154
%	65.1	69.5	67.8
oPR	56	94	150
%	65.1	66.7	66.1
# E. transf.	101	145	246
# Implant.	62	100	162
IR %	61.4	69.0	65.9

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Blastocyst Outcome per day of development and tested vs euploid after a one-step rapid-warming protocol of 1min in 1M TS

Table 7: Day 5/6/7 Blastocysts Outcome [pos. ß-hCG, cPR, oPR, IR] per age after a one-step warming protocol of 1min in 1M TS										
	Day 5 Total	Day 5 untested	Day 5 euploid	Day 6 Total	Day 6 untested	Day 6 euploid	Day 7 Total	Day 7 untested	Day 7 euploid	Total
n	114	46	68	108	39	69	5	1	4	227
pos.	88	33	55	79	29	50	4	1	3	171
%	77.2	71.7	80.9	73.1	74.4	72.5	80.0	100.0	75.0	75.3
cPR	84	31	53	67	24	43	3	1	2	154
%	73.7	67.4	77.9	62.0	61.5	62.3	60.0	100.0	50.0	67.8
oPR	82	31	51	65	24	41	3	1	2	150
%	71.9	67.4	75.0	60.2	61.5	59.4	60.0	100.0	50.0	66.1
# E. transf.	121	51	70	118	49	69	7	1	6	246
# Implant.	88	34	54	71	28	43	3	1	2	162
% IR	72.7	66.7	77.1	60.2	57.1	62.3	42.9	100.0	33.3	65.9

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Blastocyst Outcome per age and untested vs euploid after a one-step rapid-warming protocol of 1min in 1M TS

Table 8: Day 5/6/7 Blastocysts Outcome [pos. ß-hCG, cPR, oPR, IR] per age and untested vs euploid after a .ne-step warming protocol of 1min in 1M TS

	<35 [no PGT]	<35 [PGT]	35-37 [no PGT]	35-37 [PGT]	38-40 [no PGT]	38-40 [PGT]	>40 [no PGT]	>40 [PGT]	Total
n	40	43	19	33	11	37	16	28	227
pos.	32	36	16	25	7	27	8	20	171
%	80.0	83.7	84.2	75.8	63.6	73.0	50.0	71.4	75.3
cPR	30	34	14	24	6	25	6	15	154
%	75.0	79.1	73.7	72.7	54.5	67.6	37.6	53.6	67.8
oPR	30	33	14	24	6	23	6	14	150
%	75.0	76.7	73.7	72.7	54.5	62.2	37.5	50.0	66.1
# E. transf.	46	44	19	34	14	38	21	30	246
# Implant.	33	36	14	24	8	25	7	15	162
% IR	71.7	81.8	73.7	70.6	57.1	65.8	33.3	50.0	65.9

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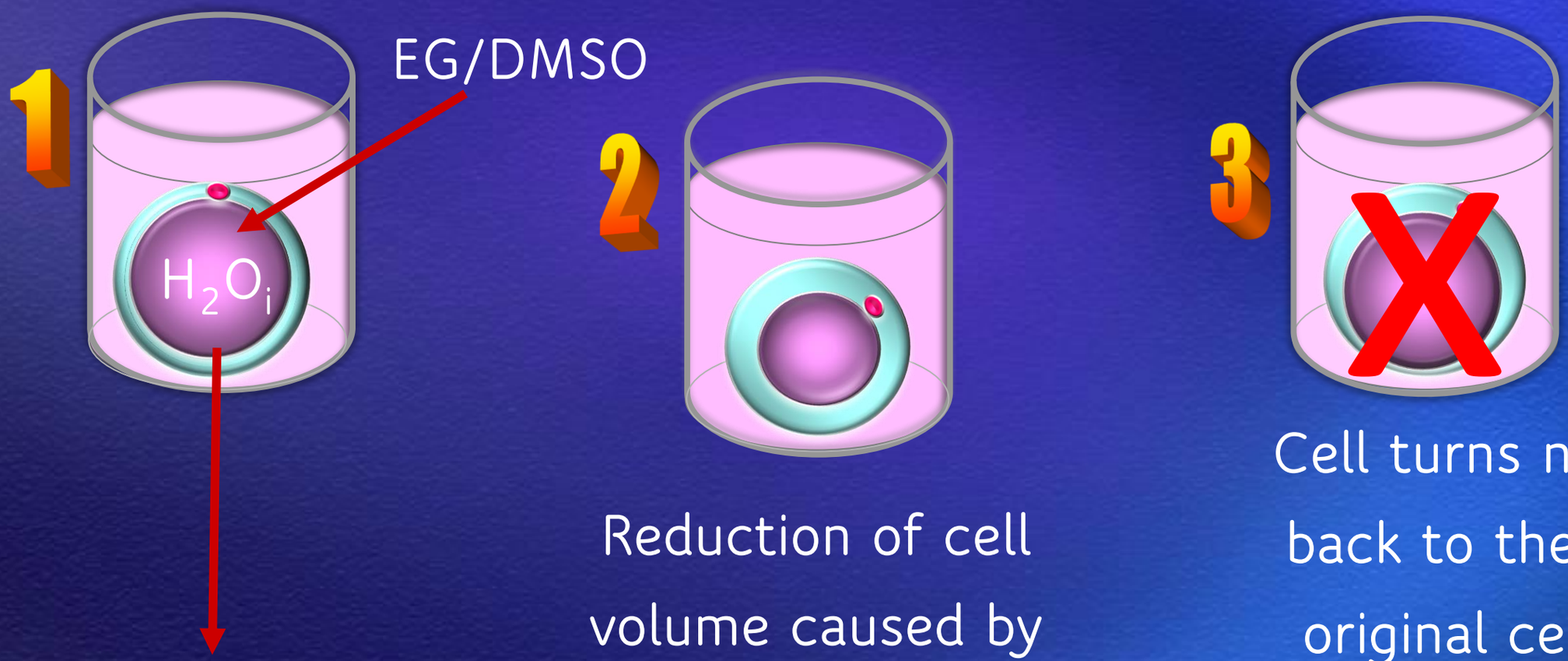
What is differ during rapid vitrification?

Higher extracellular osmolarity

Equilibrium between intra- & extracellular osmolality



Reduction of cell volume caused by dehydration

Cell turns not back to their original cell volume

We are not waiting for equilibrium and re-expansion.

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Summary on Rapid Warming

- Simplifies laboratory technique for warming blastocysts
- Reduced unnecessary time blastocysts are exposed to room temperature and in turn reduces stress to the blastocysts.
- D. Gardner showed that adding Antioxidants to the vit/warming solution is increasing the cell number in blastocysts (RBMOnline 44; 2022)

- Time-saving, which reduces pressure/stress for embryologists especially in big programs with 10 and more FETs per day. Before we always put two embryologists on thawing in case of 10 FETs; now they are all done by one.
- Blastocysts vitrified in 2min and warming in 1min in 1M sucrose but not 0.5M sucrose do survive. 2min exposure to ES/VS feels like sufficient.

- Having a kit available with 1vial ES, 1vial VS, and 1vial TS is cost saving and streamlines ordering.
- (Rapid vitrification) / rapid warming is easy to implement in your daily routine, and standardized blastocyst cryopreservation and its outcome even more successful.

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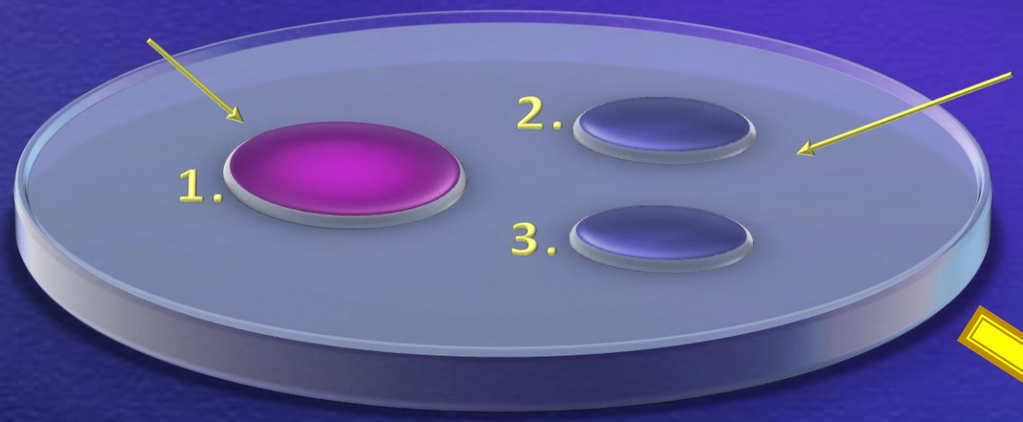
Rapid vitrification combined with rapid warming for human blastocysts

(takes 2mins for vit, and 1min for warming); thats right: total 3mins

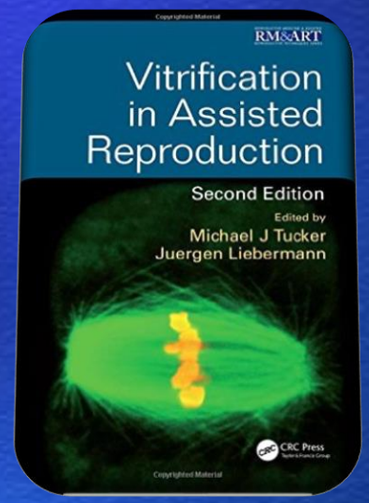
1x drop of ES
(200µl)

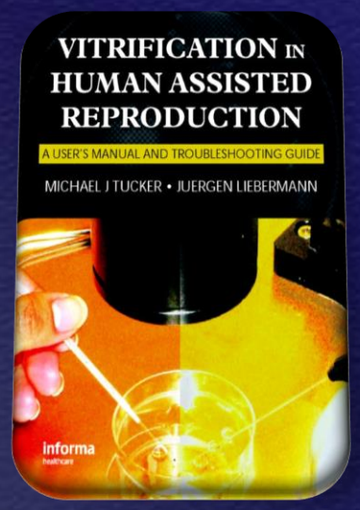
Stay for 1min



2x drops of VS
(100µl each)

Stay for 1min





1min in 400µl TS (37°C) on a 90 mm Dish Lid surface, then straight to the transfer dish with SBM and 20% SPS

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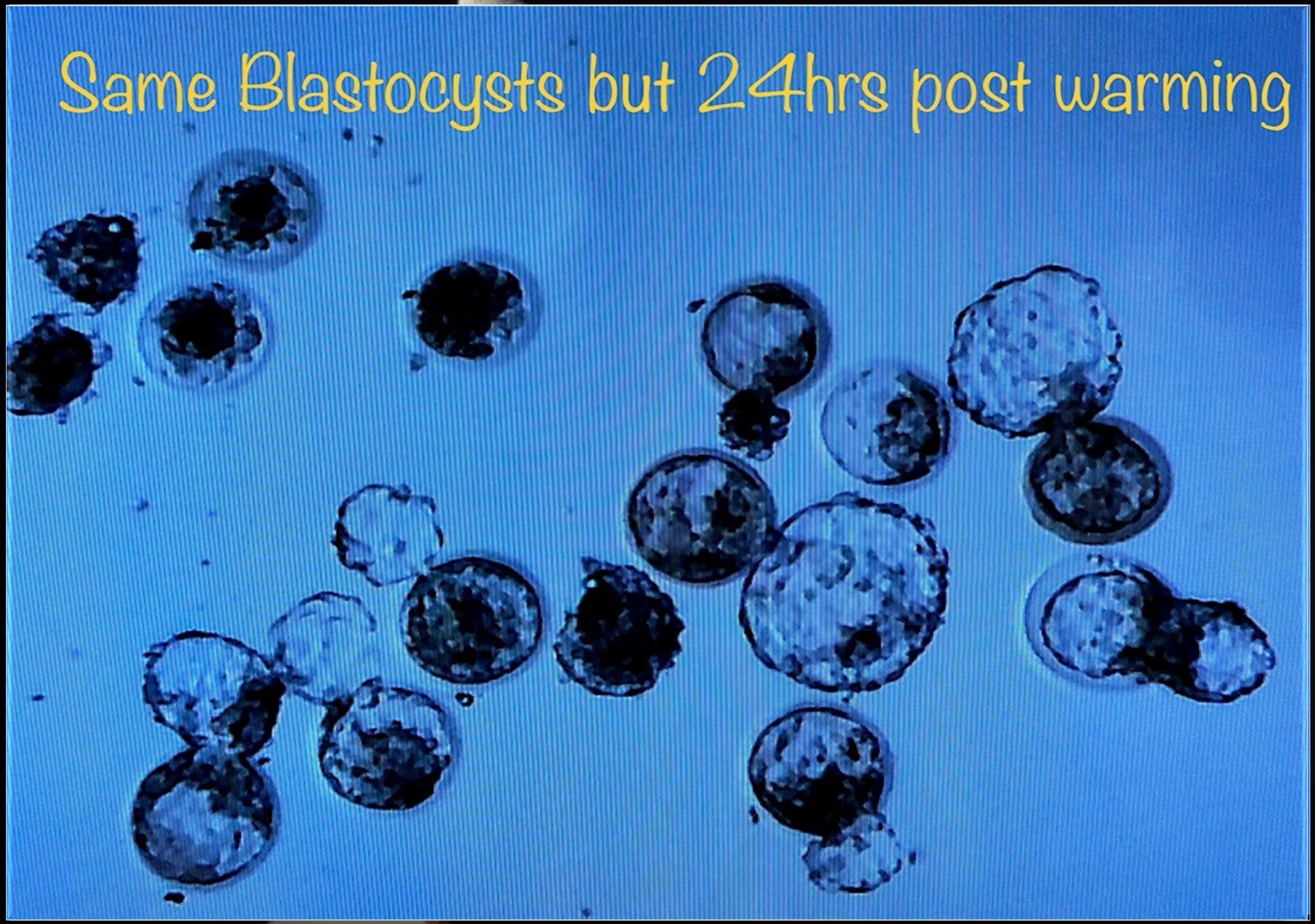
FIRST TRIAL



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In 2022

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

Is it entirely true?

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- Oocytes' osmotic behavior indicate that the dehydration upon exposure to standard CPA solutions occurs very fast: the point of minimum volume of the shrink-well curve is reached within 60 seconds

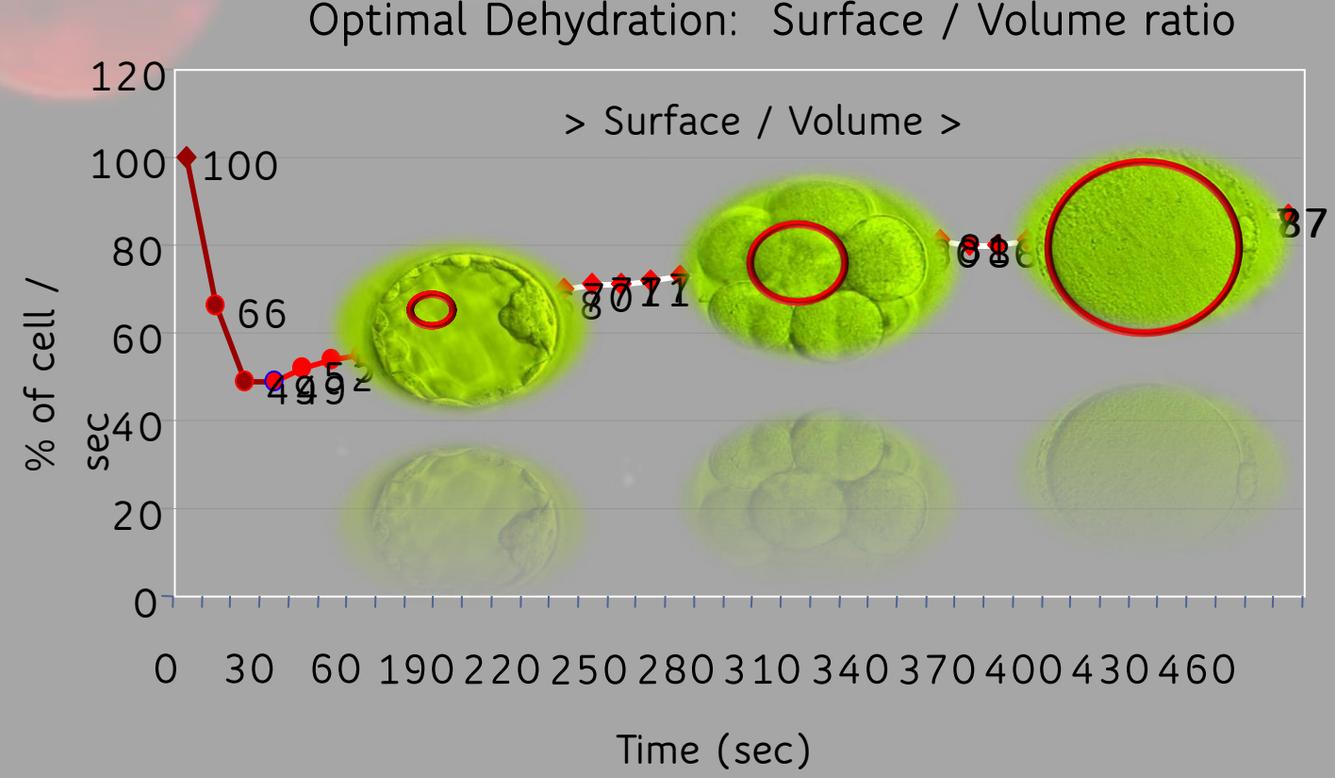
- At this point, intracellular water ejection is complete, which coupled with the permeation of low molecular weight CPA results in similar intra- and extracellular solute concentration (isotonic or equilibrium)

- 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

Gallardo & Risco, 2019
in Scientific Reports

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



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Survival of different stages of oocyte maturation after 2min of vitrification and 1min warming

Table 1: Survival of different stages of oocyte maturation after vitrification					
	GV	MI	MII	3PN	Total
n	21	41	106	10	178
Survival post warming (%)	19 (90.5)	39 (95.1)	95 (90.0)	10 (100)	163 (91.5)
Survival 24hrs post warming (%)	19 (90.5)	39 (95.1)	95 (90.0)	10 (100)	163 (91.5)

For Table 2: Cryotop or Cryolock were used as carrier and Irvine or Kitazato; no difference in survival was observed in between carriers and warming solutions

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Time-saving on a 2min vit, and 1min warming protocol

The Rapid vitrification/warming protocol demonstrates excellent survival post 24hrs warming. It's a huge time-saving protocol per week, month or annually.

	Vitrification		Warming			
	Traditional	Fast	Traditional	Fast	Traditional	Fast
N warming	Per vitrified case		Per warming case		Weekly	
1	16min	2min	10min	1min	Daily	
10	160min	20min	100min	10min	Weekly	
400	6400min	800min	4000min	400min	Yearly	

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A 2 min exposure to vitrification solutions, followed by a 1 min exposure to a warming solution delivers high survival rates for GV, MI, MII, and abnormal fertilized Pronuclei as well for cleavage stage embryos derived from 3PN.

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Patients matters:

“Our healthy baby boy was born last week!

You turned our difficult situation into a blessing beyond measure, and we are forever grateful to you. Thank you for all you do, for us and so many others.

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