







In collaboration with:







# Focus on details Continuous improvement in IVF

# The need to revisit KPIs in cryopreservation: concerns and considerations

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### **Declarations-No Conflict of Interest**

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- •Honorary Associate Professor Institute for Women's health University College London
- •Member of the Supervisory Board of the Greek National Authority of Assisted Reproduction
- •Founder and Director of the International Advanced Course in Clinical Embryology
- •Fertility Education initiative ESHRE Board Committee Member representing Greece
- •ESHRE Assessor for Centre Accreditation of Training Program in Clinical Embryology International
- •Director of the MSc in Applied and Molecular Physiology with a specialization in Clinical Embryology and Genetics of the Preimplantation Embryo
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- •Inspector for Temos Accreditation Healthcare Center for excellence in Reproductive Care
- •Associate Editor of Human Repoduction Update, Member of the Editorial Board JARG and SysBirm

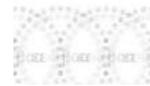














**Oocyte Retrieval** 

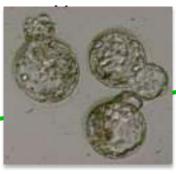








Assisted

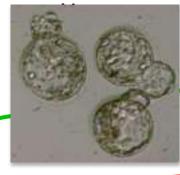


Cleavage Assessment

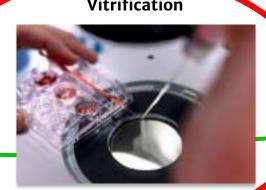


**Embryo Transfer** 





Vitrification



Warming



Trophectoderm Biopsy

Adapted from Professors Bormann and Dimitrio

# Cryopreservation in Europe ... On the rise

> Hum Reprod. 2023 Dec. 4;38(12):2321-2338, doi: 10.1093/humrep/dead197.

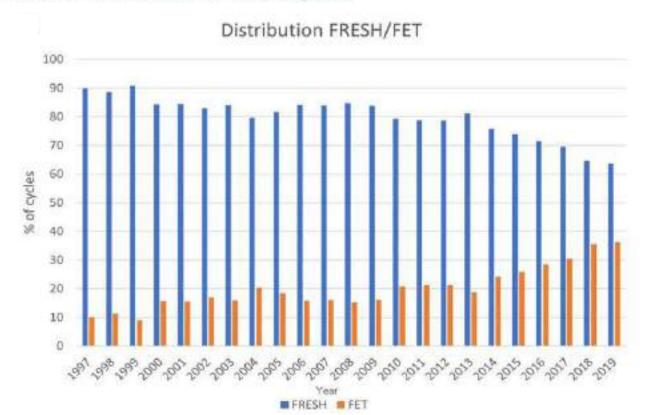
## ART in Europe, 2019: results generated from European registries by ESHRE†

European IVF Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE);

Jesper Smeenk <sup>1</sup>, Christine Wyns <sup>3</sup>, Christian De Geyter <sup>3</sup>, Markus Kupka <sup>4</sup>, Christina Bergh <sup>5</sup>, Irene Cuevas Salz <sup>6</sup>, Diane De Neubourg <sup>7</sup>, Karel Rezabek <sup>8</sup>, Andreas Tandler-Schneider <sup>9</sup>, Ionna Rugescu <sup>10</sup>, Veerle Goossens <sup>15</sup>

Collaborators, Affiliations + expand

PMID: 37847771 PMCID: PMC10694409 DOI: 10.1093/humrep/dead197



	FEI						
Country	Thewings FET	Pregnancies per thanking (%)	Deliveries per disawing (14				
Albarra	71	27.4	30.5				
Armenia	2143	38.0	32.8				
Austrie	5485	39.1					
Belgras	1336	37.7	243:				
Beigium	74.597	78.6	729.39				
Bornis Demigorita, Pederation port	21	22.5	15.5				
Bulgatia	1872	40.2	33.0				
Czech Republic Dennazik	25 174	31.0	18.9				
Estoria Poland	1142	30.1	22.3				
France	45 312	25.4	21.2				
Cormany	30 436	28.8	237.5				
Greece	5602	44.9	28.3				
Hungary	1616	24.3	18.3				
feeland	377	42.4	33.2				
Ireland	814	42.5	32.2				
Huly.	22, 256	31.0	20.2				
Karakhatan	4860	46.0	35.0				
Canda	551	44.3	20.6				
Lithuania	387	48.3	7.2				
Lonembourg	· O		2000				
Multa	23	39.1	30.4				
Moldova	415	33.7	24.8				
Morephages	75	41.3	38.7				
North Macedonia	400	46.1	20.9				
Norway	4516	28.0	23.2				
Poland	12.875	337.5	25.6				
Portugal	3125	27.0	25.6				
Rigoria	30.864	42.7	27.0				
Szitlia	465	24.1	15.3				
Skyaka	300						
Siovensa	1721	35.6	26.7				
Spain	35 357	32.5	26.5				
	200	200	ALE CO.				
Sweden Switzerland The Netherlands	5524	24.4	24.8				
	8433	49.5	39.1				
Turber	18.892	50.1	41.4				
Ukraine	24.300	1945.4	74.00				
All	309 311	32,9	22.7				

FEE



70.200 deliveries



#### THE MAIN STEPS IN THE HISTORY OF OOCYTE AND EMBRYO CRYOPRESERVATION



#### STEWART INC.

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BUREF WA

First birth with harmon

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**AVERILL AND ROWSON** 

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305 - 405

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705

Curvival of classage mage

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The two feature hypothesis of CHEST AND LABOUR.

#### MAGILIAN YAS

The cocyte melatic spindle is sensitive to temperature

#### TROUNSON WITE

first birth using embryon recent in a DMSO solution.

#### MENAND 1994

Hammakan probryos successfully traper in PRCH

#### LINSTRALLS THREE

The Locate procedure

805

RALL AND FAHY 1985

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First histoire boin after

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#### ETELECHNUS 1990

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#### SHAW HES

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#### ALT AND SHELTON 1003

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F996-2000: sunformer brother PROPERTY AND ARREST PROPERTY.

#### WICOD 1933

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#### GOCK 1991, 1994, 1995

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#### BIELAMERS 2000

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#### CARREST THEFT

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#### MICKARDA 3001

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#### LIEBERMANN 2002

The feeiget carrier (upen)

#### AND TUCKER JOSS

The homi-strew samer jopens

Live sarth offer cryopresentation in challes based medium

#### VANDEREWALMEN 2005

Live birth from a bisetoryet vitation with the hemi-strew commen

005

KATAYAMA 2003

The Natayama protocol

IEG + DM90 + sucroses

KLIWAYANA 2005

Cryotip camer (closed system)

CHINE 3000

ECPPROMY NUCLOSE VICINICATION

on Crycleaf carrier (spert)

RUWAYANA 3005

The nuveryama protocol

(ED-DMSO+microssion Cryotop sarrier (open)

LADMAN SOOT

The meknic spindle is

preserved during human and mouse docyte vitaffication

and warming

High survival and healthy

deliveries with the Cryotco

medhad

#### PARMEDIAN 2010

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#### COBO 2010

thorage of open cargon. In the vagor phase of limit authorgen to resture any transmental continuous

#### GANCIA RING

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#### MINASI 2003

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#### CUTHISKY 200

The cryobacti carrier

#### **DESAL 2013**

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Live birth with a cryopeniones human Ca

#### STACHECKI 1998

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#### ALESHOVA 1989

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#### LANCTON.

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#### PITME 2014

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#### THE REAL PROPERTY.

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#### ARRY 35H

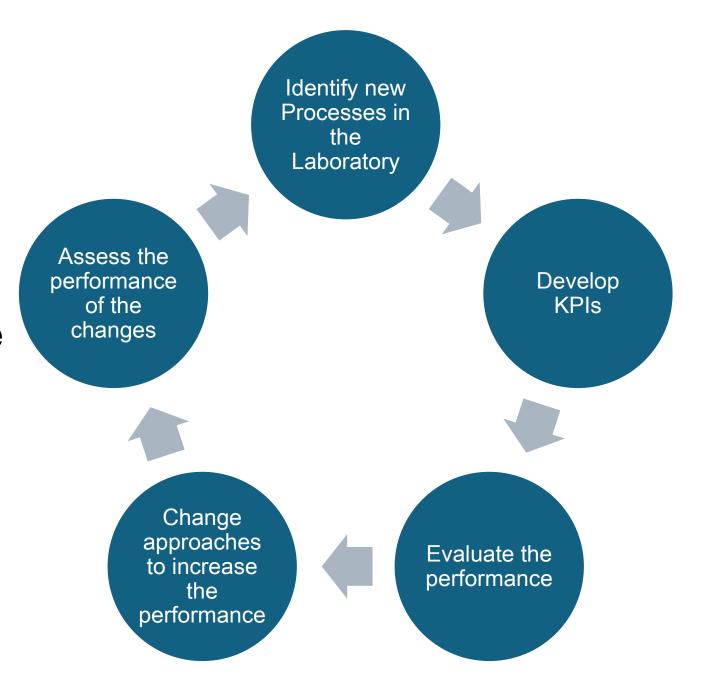
Saruty for automatic eroboyo attellication

EMBRYOLOGY GROUP

### Occyte and embryo cryopreservation in assisted reproductive technology: past achievements and current challenges Casciani, Valentina et al. Fertility and Sterility, Volume 120, Issue

# What are the KPIs

- Key Performance Indicators (KPIs) are employed to evaluate and measure our performance
- KPIs are not static but a dynamic process
- Positive reinforcement cycle



> Reprod Biomed Online. 2012. Aug;25(2):146-67. doi: 10.1016/j.rbmo.2012.05.006. Epub 2012. May 29.

# The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting

Alpha Scientists In Reproductive Medicine

# Oocyte

KPt			Competence	Benchmark
01	Morphological	Freezing	≥50%	75%
0.1	survivol	Vitrification	yo46	85% (95% for donors <30 years)
0z	Fertilization rate		10% (absolute; i.e. so percentage points) lower the fresh occytes at the centre	on that for the comparable
01	Embryo development rate	Freezing	No more than 10–30% (relative) lower than that for the comparable population of fresh embryos at the contre	The same as for the comparable population of fresh embryos at the centre
		Vitrification	The same as for the comparable population of	fresh embryos at the centre
04	Implontation rate	No more than the centre	10-30% (relative) lower than that for the compara	this population of fresh embryos at

# Cleavage stage embryo

KPI			Competence	Benchmork
E.	Morphological survival: fully	Freezing	40%	55.56
£i.	intect	Vitrification	70%	85%
£2	Morphological survival: >50%	Freezing	63%	85%
	intoct	Vitrification	85%	95%
f3	Post-thow development (including implantation rate) for fully intoct embryos		<ao% (relative)="" for<br="" lower="" than="" that="">the comparable population of fresh embryos at the centre</ao%>	The same as for the comparable population of fresh embryas at the centre

# Blastocyst

KPI			Competence	Benchmark
Ві	Survival rate	Freezing	70%	85%
		Vitrification	80%	95 <sup>9</sup> 6
Ba .	Transfer rate	Freezing	Joge	85%
		Vitrificution	80%	059n
83	Implantation		<to% (relative)="" at="" centre<="" comparable="" embryos="" for="" fresh="" lower="" of="" p="" population="" than="" that="" the=""></to%>	The same as for the composable population of fresh embryos at the central

Table IV KPIs for the ART laboratory.

KPI	Calculation	Competency value (%)	Benchmark value (%)
ICSI damage rate	$rac{ ext{no. damaged or degenerated}}{ ext{all oocytes injected}}  imes 100$	≤10	≤5
ICSI normal fertilization rate	$rac{ ext{no. oocytes with 2PN and 2PB}}{ ext{no. MII oocytes injected}}  imes 100$	≥65	≥80
IVF normal fertilization rate	$rac{ ext{no. cocytes with 2PN and 2PB}}{ ext{no. COCs inseminated}}  imes 100$	≥60	≥75
Failed fertilization rate (IVF)	$\frac{\rm no.cycles~with~no~evidence~of~fertilization}{\rm no.of~stimulated~IVF~cycles}\times100$	<5	
Cleavage rate	no . cleaved embryos Day 2 no . 2PN/2PB oocytes on Day 1 $\times$ 100	≥95	≥99
Day 2 Embryo development rate	$\frac{\text{no.4-cell embryos on Day 2}}{\text{no. normally fertilized oocytes}^a}\times 100$	≥50	≥80
Day 3 Embryo development rate	$\frac{\text{no. eight cell embryos on Day 3}}{\text{no. normally fertilized oocytes}^3}\times 100$	≥45	≥70
Blastocyst development rate	$\frac{\rm no}{\rm no}$ . blastocysts Day 5 $_{\rm no}$ . normally fertilized oocytes $^{\rm a}$ $\times$ 100	≥40	≥60
Successful biopsy rate	$rac{ ext{no. biopsies with DNA detected}}{ ext{no. biopsies performed}}  imes 100$	≥90	≥95
Blastocyst cryosurvival rate	$rac{ ext{no. blastocysts appearing intact}}{ ext{no. blastocysts warmed}}  imes 100$	≥90	≥99
Implantation rate (cleavage-stage) <sup>b</sup>	$rac{ ext{no. sacs seen on ultrasound}^c}{ ext{no. embryos transferred}}  imes 100$	≥25	≥35
Implantation rate (blastocyst-stage)	$rac{ ext{no. sacs seen on ultrasound}^c}{ ext{no. blastocysts transferred}}  imes 100$	≥35	≥60

- In ESHRE's KPIs (Vienna Consensus) only blastocyst cryosurvival rate was included as a KPI
- The cryosurvival was evaluated according to morphological appearance
- Would that introduce a certain degree of relativity?

# The Vienna consensus: report of an expert meeting on the development of art laboratory performance indicators \*\* 3

ESHRE Special Interest Group of Embryology, Alpha Scientists in Reproductive Medicine Author Notes

Human Reproduction Open, Volume 2017, Issue 2, 2017, hox011,

https://doi.org/10.1093/hropen/hox011

Published: 04 August 2017 Article history v

# Even in 2023, looks still matter...



J Assist Reprod Genet, 2023 Apr 24;40(6):1479-1494, doi: 10.1007/s10815-023-02792-1 ttt

Clinical and laboratory key performance indicators in IVF: A consensus between the Italian Society of Fertility and Sterility and Reproductive Medicine (SIFES-MR) and the Italian Society of Embryology, Reproduction and Research (SIERR)

Alberto Vaiarelli <sup>1,83</sup>, Cariotta Zach <sup>2,8</sup>, Valentina Spadoni <sup>2</sup>, Danilo Cimadomo <sup>1</sup>, Alessandro Conforti <sup>3</sup>, Carlo Alviegi <sup>4</sup>, Bubuno Palermo <sup>5</sup>, Carlo Bulletti <sup>6,7</sup>, Lucia De Santis <sup>8</sup>, Valerio Pisaturo <sup>9</sup>, Vincenzo Vigiliano <sup>10</sup>, Giulia Scaravelli <sup>10</sup>, Fišopo Maria Ubuldi <sup>1</sup>, Andrea Borini <sup>2</sup>

Table 2 List of PIs identified by SIFES-MR and SIERR panel of experts with medium agreement (40-80%). Competence and benchmark values were proposed for each KPI, along with a suggested frequency for their analysis

PERFORMANCE INDICATORS	Reference population	Competence value	Benchmark value	Suggested frequency of analy Monthly or 100 cycles		
IVF fertilization rate	All patients (except for SMF)	≥60%	≥75%			
2. Oocytes cryo-survival rate	All patients	≥70%	≥85%	Monthly or 100 cycles		
<ol> <li>Embryo cryo-survival rate: &gt; 50%</li> </ol>	All patients	≥80%	≥95%	Monthly or 100 cycles		
blastomeres survived AND 3. Embryo cryo-survival rate: all blastomeres survived		≥70%	≥85%			
4. Blastocyst cryo-survival rate	All patients	≥90%	≥99%	Monthly or 100 cycles		
5. Successful biopsy rate	All patients	≥95%	≥97%	Monthly or 100 cycles		
6. Follicular output rate (FORT)	All patients	≥40%	≥80%	Monthly or 100 cycles		
7. Cumulative live birth rate (CLBR)	a)≤34 years	a)≥30%	a)≥40%	Yearly		
	b) 35-39 years	$b)\!\geq\!20\%$	b)≥30%			
	c)≥40 years	c)≥5%	c)≥10%			

#### 2. Oocyte cryo-survival rate

- Definition: Proportion of morphologically intact oocytes at the time of ICSI after thawing-warming.
- 3. Embryo cryo-survival
- Definition: Survival was defined as the proportion of thawed-warmed viable embryos with at least 50% blastomeres intact and with all blastomeres intact.
- 4. Blastocyst cryo-survival
- Definition: Blastocyst cryo-survival was defined as at least 75% of cells intact after thawing-warming

- Is morphologically assessed "survival" enough?
- How is the threshold of "at least 50% or 75% intactness decided on?"
- What does survival mean?
- Does intactness =survival?

# Anything else worthwhile reporting on?

Table 3. Embryo kinetics of development according to the type of cycle (fresh donations and donations with the use of vitrified oocytes).

Timing (h)	Group	n	Mean (h)	95% CI	Pvalue
12	Fresh	9,935	27.7	27.6-27.8	<.01
	Vitrified	3,793	28.7	28.5-28.9	
r3	Fresh	9,746	37.8	37.6-37.9	<.01
	Vitrified	3,697	38.9	38.5-38.9	
t4	Fresh	9,546	40.2	40.1-40.3	<.01
	Vitrified	3,585	41.4	41.2-41.7	
t5	Fresh	8,789	50,5	50.3-50.7	<.01
	Virrified	3,141	51.7	51.4-52.1	
tM	Fresh	2,594	86.6	86.1-87.1	<.01
	Vitrified	779	88.5	87.5-89.4	
tB	Fresh	1,938	103.4	103-103.9	.016
	Vitrified	580	104.5	103.7-105.4	
tEB	Fresh	1,045	114.4	113.8-114.9	.466
	Vitrified	325	114.8	113.7-115.9	
tHB	Fresh	164	114.9	113.4-116.4	.196
	Vitrified	59	116,9	113.8-120	
cc2	Fresh	9,746	10.2	10.1-10.3	.901
	Vitrified	3,697	10.2	10.0-10.4	
s2	Fresh	9,546	2.6	2.5-2.6	<.01
	Vitrified	3,585	2.9	2.7-3.1	

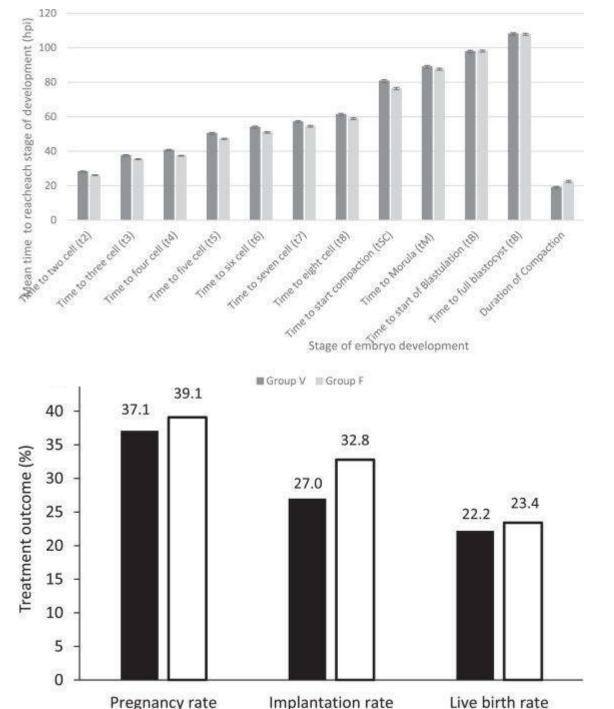
### What about time?

Observational Study > Fertil Steril. 2017 Sep;108(3):491-497.e3. doi: 10.1016/j.fertnstert.2017.06.024.

## Effect of oocyte vitrification on embryo quality: time-lapse analysis and morphokinetic evaluation

Ana Cobo <sup>1</sup>, Alla Coello <sup>2</sup>, Jose Remohí <sup>2</sup>, Jose Serrano <sup>2</sup>, Jose Maria de Los Santos <sup>2</sup>, Marcos Meseguer <sup>2</sup>

Until Blastocyst formation, embryos deriving from frozen oocytes seem to develop slower than embryos derived from fresh oocytes.



### More recent data

Multicenter Study > Reprod Biomed Online, 2023 Jul;47(1):51-60. doi: 10.1016/j.rbmo.2023.02.011. Epub 2023 Mar 1.

A comparison of the morphokinetic profiles of embryos developed from vitrified versus fresh oocytes

Kathryn Montgomery 1, Susan Montgomery 2, Alison Campbell 3, Deborah Mary Nash 1

Similar results to previous data indicating slower rates up to compaction.

However, time to blastocyst formation was similar between embryos deriving from fresh and frozen oocytes.

No statistically significant difference on live-birth rates. Some difference in implantation rates

# Very recent data

J Assist Reprod Genet, 2024 Mar;41(3):643-648, doi: 10.1007/s10815-023-03016-2.
 Epub 2024 Jan 11.

Similar pregnancy outcomes from fresh and frozen donor oocytes transferred to gestational carriers: a SART database analysis isolating the effects of oocyte vitrification

Tacqueline Kloca <sup>1</sup>, Channing fiurks <sup>2</sup>, Alexandra Purdue-Smithe <sup>3</sup>, Elizabeth DeVillbiss <sup>4</sup>, Sunni L Mumford <sup>5</sup>, Rachel Weinerman <sup>6</sup>

Outcome	Fresh donor oocyte and gestational carrier (n = 1119)	Frozen donor oocyte and gestational carrier (n = 165)	RR (95% CI)	aRR (95% CI)*
Pregnancy Outcomes, n (%)				
Live birth	534 (47.7)	76 (46.1)	0.97(0.81, 1.15)	0.89 (0.70, 1.13)
Clinical pregnancy	611 (54.6)	90 (54.5)	1.00 (0.86, 1.16)	1.01 (0.82, 1.24)
Biochemical pregnancy loss	66 (5.9)	17 (10.3)	1.75 (1.05, 2.9)	1.00 (0.46, 2.19)
Clinical pregnancy loss	132 (11.8)	29 (17.6)	1.49 (1.03, 2.15)	1.13 (0.65, 1.97)
Birthweight, n (%)				
Low birthweight (< 2500 g)	190 (26.6)	22 (23.7)	0.92 (0.63, 1.35)	0.85(0.52, 1.39)
Normal birthweight (≥ 2500 g and ≤ 3999 g)	480 (67.2)	62 (66.7)	Ref	Ref
High birthweight (> 4000 g)	44(6.2)	9 (9,7)	1.51 (0.77, 2.96)	0.50 (0.14,
Pregnancy plurality, n (%)				
Singleton	341 (63.9)	58 (76.3)	Ref	Ref
Multiple	193 (36.1)	18 (23.7)	0.66 (0.43,1)	0.77(0.47, 1.29)

Use of frozen oocytes
in gestational carriers
provided similar
clinical outcomes
with fresh oocytes in
gestational carriers
which may serve as a
KPI

Cochrone Database of Systematic reviews. Review - Intervention



#### Fresh versus frozen embryo transfers in assisted reproduction

🌉 Tjitske Zaat, Mirium Zagors, Fornke Noi, Mariëtte Goddijn, Madelon van Wely, Sebastiaan Mastenbroek

Authors' declarations of interest

Wester published: D4 February 2021. Version history

Mtps://doi.org/10.1002/14651056.00011184.pub/15P

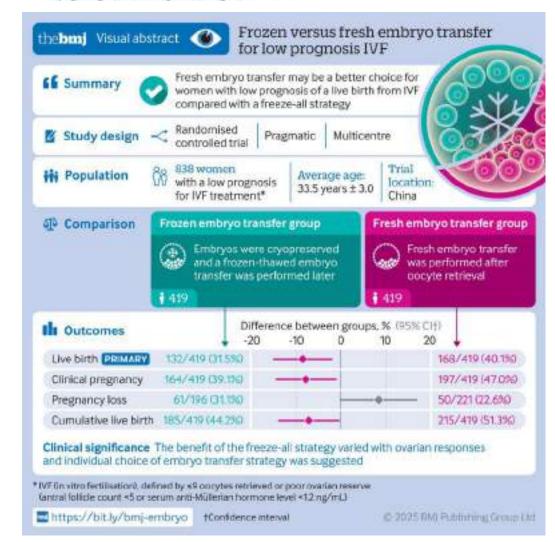
	Freeze	all IVF	Convention	onal IVE		Odds Ratio	Odds Ratio		R	isk	of B	125	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI	A	В	C	D	F	6
1.1.1 Live birth rate:	cumulativ	ely for ck	eavage sta	ge transf	er								
Chen 2016	465	746	455	762	34.1%	1.12 [0.91 , 1.37]							1
Ferraretti 1999	23	58	26	87	2.9%	1.04 [0.50 , 2.13]	1	2	3	٠		0 7	
Visiong 2018	191	391	185	391	19.0%	1.06 (0.80 , 1.41)			3				1
Subtotal (95% CI)		1195		1220	56.0%	1.09 [0.93 , 1.29]	•						
Total events:	679		866				18						
Haterogeneity: Chit =	0.10, df = 2	P = 0.9	5); 1" = 0%										
Test for overall effect:	Z = 1.09 (P	=0.28)											
1.1.2 Live birth rate:	cumulatiw	ely for bl	astocyst st	age trans	ster								
Santos-Ribeiro 2020	65	90	67	94	3.7%	1.05 [0.55 , 1.99]							
Shapiro 2011a	37	70	35	67	3.4%	1.03 [0.52 , 2.00]		2		ō	•		10
Snapiro 2011b	37	60	39	82	3.0%	0.95 [0.46 , 1.97]		2		ō			
Wei 2019	627	825	604	825	29.1%	1.16 [0.93 , 1.45]							19
Wong 2021	18	102	29	102	4.8%	0.54 [0.28 , 1.05]	77 = 22			•			10
Subtotal (95% CI)		1147		1150	44.0%	1.08 [0.88 , 1.27]	•	-	-				
Total events:	784		774				100						
Halerogeneity: Chir =	4.65, df = 4	(P = 0.3	2): 1" = 14%										
Test for overall effect:	Z = 0.59 (F	-0.55											
Total (95% CI)		2342		2370	100.0%	1.08 [0.95 , 1.22]	<u> </u>						
Total events	1463		1440										
Heterogenetty: Chit -	4.83. df = 7	(P-05	81: 12 - 0%			7	2 95 2	5					
Test for overall effect:		THE RESERVE	5600105361				conventional Favours fre	eze-all					
Test for subgroup diffe	manage Ch	7 - 0.07	M-1/D-	0.701 17	001								

The jury is still out there regarding **embryo** cryopreservation effect on LBR

Randomped Controlled Trial > 8MIL 2025 Jan 29:388:x481474. doi: 10.1136/bmj-2024-081474.

### Frozen versus fresh embryo transfer in women with low prognosis for in vitro fertilisation treatment: pragmatic, multicentre, randomised controlled trial

Damin Wei 1 2 3 4, Yun Sun 2 5, Hen Zhoo 1 2 3 4, Junhoo Yen 1 2 1 4, Hong Zhou 1, Fei Gong 5, Ağun Zhang 9, Zu Wang 1 2 3 4, Lisi sh 10, Hongdru Bao 11, Shuyin Zhao 12, Zhuuni Xioo 11, Yingying Cin 1 2 3 4, Ling Geng 1 2 3 4, Linkn Cui 1 1 3 4, Yon Sheng 2 2 3 4, Mei Sun 1 2 3 4, Peikeo Us 1 2 3 4, Ungling Ding 1 3 3 4, Hong Liu 1 2 3 4, Yan U 1 2 3 4, Yan Li 3 2 3 4,



# Summary I

No statistically significant difference in live-birth rates comparing fresh and frozen oocytes

Vitrified oocytes seem to develop slower at least up to compaction stage

Embryo vitrification in general population seem to result to similar clinical outcomes compared to fresh embryos.

This may not be the case for all subpopulations – especially poor prognosis women. Should we consider different KPIs for different populations?



What else do we need to consider

# What about

Post-warming time

Day of blastocyst vitrification

Physiology

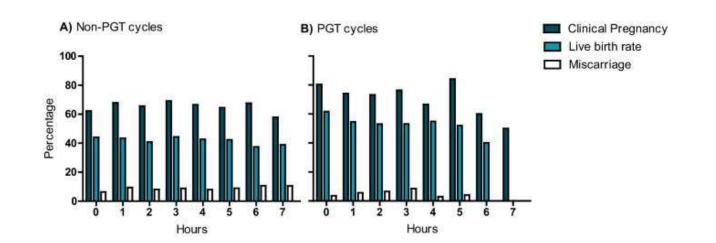
# **Blastocyst Post-Thaw Culture**

D<sub>5</sub>

Outcome	Short culture	Long culture	p Value
	(n = 3n4)	(n = 384)	
Implantation rate, n/N (%)	287/486 (50.1)	306/483 (63.4)	0,170
Clinical pregnancy rate, n/N (%)	245/384 (63.8)	257/384 (56.9)	0.363
Abortion rate, n/W (%)	29/245 (11.8)	37/257 (14.4)	0.396
Live birth rate, n/N (%)	216/384 (56.3)	230/384 (57.3)	0,771

D6

922000	Short culture	Long culture	10000	
Outcome	ome (n=544)		p Value	
Implantation rate, $\alpha(N)(\%)$	356/761 (45,6)	173/467 (35.5)	< 6.001	
Olinical pregnancy rate, n/N (%)	308/544 (56.6)	148/327 (45.3)	0.001	
Abortion rate, n/N (%)	44/308 (14.3)	32/148 (21.6)	0.049	
Live birth rate, n/V (%)	264/544 (48.5)	116/327 (35.5)	<8.001	



Syst Biol Regrad Mod. 2173 Febs:91(1):64-74. doi:10.1081/1999988.2022.2121191.
Epub 2022 Sep 21.

Influence of post-thaw culture duration on pregnancy outcomes in frozen blastocyst transfer cycles

HILLI T, Sharren Cau T, Har Dong T, Li Dong T, Chun Zhao T, Hangang Zhang T, Ang Lu T, Kuting Li T, Kuting Ling T

Short: 0-6

Long: over 7h

The difference between short and long culture is present only for D6 embryos (extended culture worse outcomes)

The JARG study shows post thaw culture between 0-7 hours no difference

J Assist Reprod Genet. 2024 Jun;41(5):1539-1547. doi: 10.1007/s10815-024-03115-8
 Epub 2024 Apr 20.

Effect of time post warming to embryo transfer on human blastocyst metabolism and pregnancy outcome

Golf Ardestan; <sup>1</sup>, Marion Martins <sup>2, 2</sup>, Olcay Ocali <sup>2</sup>, Tim H Sanchez <sup>4</sup>, Colwyn Gulliford <sup>4</sup>, C Bront Barrett <sup>2</sup>, Genny Sakkas <sup>2</sup>



# Theriogenalogy www.na.rayrkans.rogens.as



# Looking at oocyte data



Theriogenology Water ESR 1 September 2822, Fages 182-298



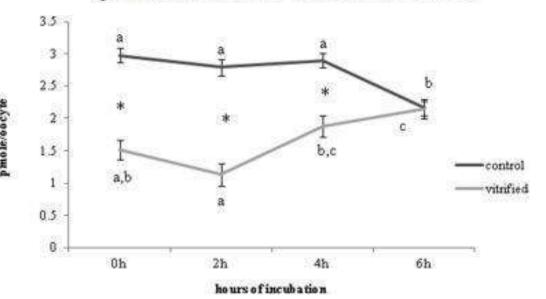
A recovery time after warming restores mitochondrial function and improves developmental competence of vitrified ovine oocytes

Sona Saczu <sup>a</sup> <sup>a</sup>, <sup>a</sup> <sup>b</sup>, Sengio Domenico Gaccu <sup>a</sup>, Bisa Sano <sup>a</sup>, Angelo Zinaliu <sup>b</sup>, Ciribco Comu <sup>b</sup>, Cristian Porcu <sup>a</sup>, Salvetere Nottena <sup>a</sup>, Flori metta Sartinguar <sup>a</sup>, Giovanni Giuseppe Leani <sup>a</sup>

### Recovery of spindle morphology and mitochondrial function through extended culture after vitrification-warming of bovine oocytes

Timil to Gutterno-Concillo 25 , Fabian A. Dian 22 , Sydney A. Talbot 22 , Kenneth R. Bondicki Å. 25

# Quantification of ATP intracellular content



Mitochondrial function as measured by ATP content seems to be similar to fresh oocytes following 6 hours.

Table 2. Experiment 1: effect of post-warming extended culture on the meiotic spindle of vitrified bovine oocytes.

Treatment	N	Mat. Rate	Microtubule Distribution		Chromosomal Arrangement		
	%		Normal	Abnormal	Normal	Abnormal	
Control	41	73.17ª	25 (83.3%)	5 (16.7%) <sup>a</sup>	26 (86.7%)	4 (13.3%)	
Vit.+EC	35	83.33 <sup>a</sup>	22 (73.3%)	8 (26.7%)a	25 (83.3%)	5 (16.7%)ab	
Vit. Control	34	76.47 <sup>a</sup>	11 (42.3%)	15 (57.7%)b	16 (61.5%)	10 (38.5%)b	

Similarly Extended Culture (6h vs 2h) seems to ameliorate the effects of vitrification completely on microtubule distribution and partially on chromosomal arrangement

\*Research on oocyte extended culture is mainly performed in animal models

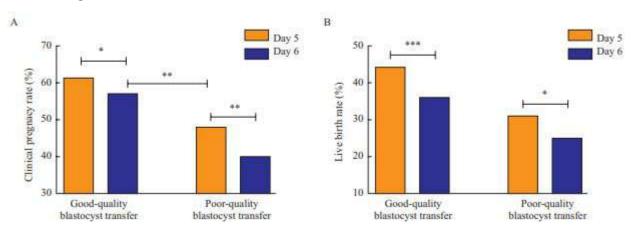
# Day of Blastocyst freezing-Does it matter? -Yes

> Curr Med Sci. 2023 Apr;43(2):297-303. doi: 10.1007/s11596-023-2699-4. Epub 2023 Mar 17.

Pregnancy Outcomes for Day 5 Versus Day 6 Single Frozen-thawed Blastocyst Transfer with Different Qualities of Embryos: A Large Matched-cohort Study

Qiong Yu 1, Hui He 1, Xin-Ling Ren 1, Shi-Fu Hu 2, Lel Jin 3

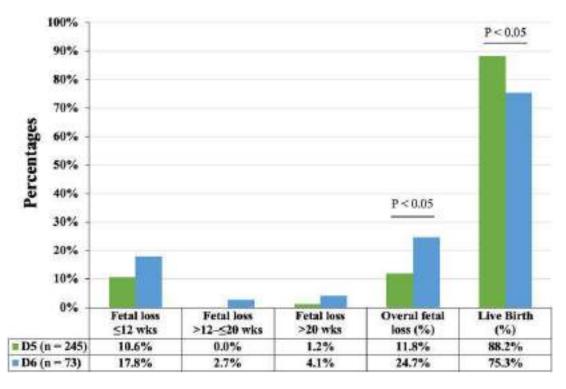
D5 seems to provide better results
Different KPIs needed or freeze only on
D5?



Increased incidence of live births in implanted day 5 versus day 6 blastocysts following single embryo transfers with PGT-A

Chien Hong Chen Chun Lles. Chun Chia Huang, Haiu Hai Chen, Chih Ying Chang, En Hai Cheng, Pin You Lin, Chung-L Chen, Tsung-Haien, Lee St. & Mass-Steng Lee St.

Scientific Reports 13, Article number: 12725 (2023) | Gite this article



Cryopreservation on D5 provides enhanced CPR and LBR compared to D6 both in good and in poor quality embryos—and in PGT-A tested embryos



Do we see everything? Are things really as they appear...or can looks be deceiving?

Do we see everything?

Reviews and Hypotheses

Mammalian pre-implantation chromosomal instability: species comparison, evolutionary considerations, and pathological correlations

Lucia Carbone & Shewn L. Chavez 35

ages 301-305 | Received 35 May 2015, Accepted 11 Jun 2015, Published unline: 14 Sey 2015

Developmental stage

Time-lapse imaging

Predictive parameters

Epigenetic reprogramming

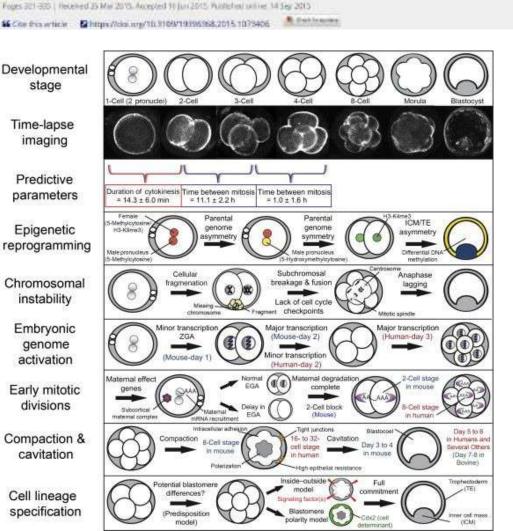
Chromosomal instability

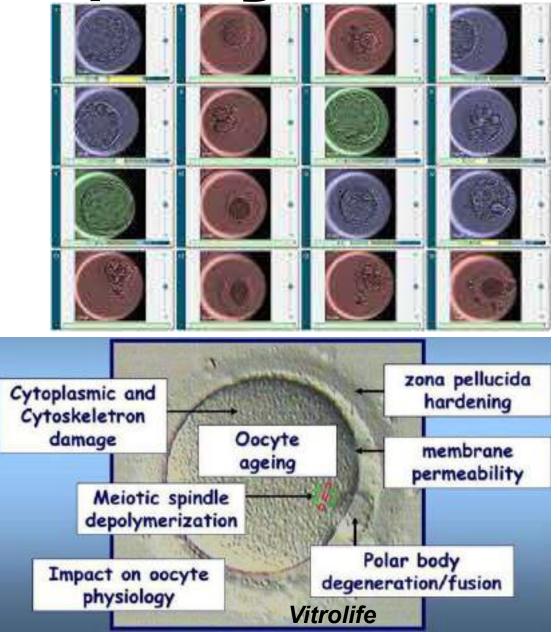
> Embryonic genome activation

Early mitotic divisions

Compaction & cavitation

Cell lineage specification



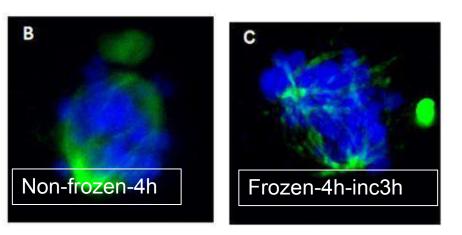


Cryobiology, 2021 Jun;100;110-116, doi: 10.1016/j.cryobiol.2021.03.001, Epub 2021 Mar 5.

# Important parameters affecting quality of vitrified donor oocytes

Olga Teplá <sup>1</sup>, Zinovij Topurko <sup>2</sup>, Jaromír Mašata <sup>3</sup>, Simona Jirsová <sup>4</sup>, Michaela Frolíková <sup>5</sup>, Kateřina Komrsková <sup>6</sup>, Adela Minks <sup>7</sup>, Jaroslav Turánek <sup>8</sup>, Anna Lynnyk <sup>9</sup>, Irena Kratochvílová <sup>18</sup>

In 3D reconstructions, the average **volume of the chromosomes from the vitrified oocytes** was more than **1.8 times greater** than the average volume of the chromosomes from the never-frozen oocytes.

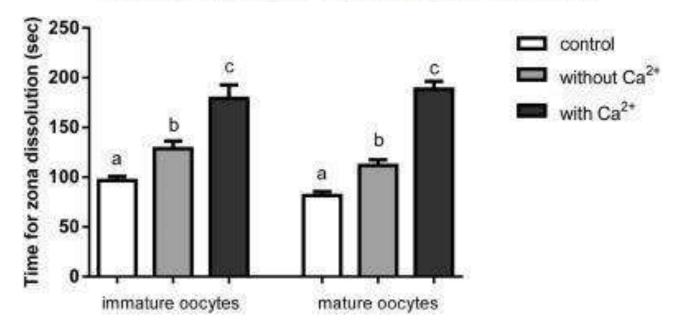


# We need to understand more and better

> Cryobiology. 2017 Jun:76:18-23. doi: 10.1016/j.cryobiol.2017.05.001. Epub 2017 May 6.

Effect of vitrification on the zona pellucida hardening and follistatin and cathepsin B genes expression and developmental competence of in vitro matured bovine oocytes

Teresa Wiesak 1, Marta Wasielak 2, Aleksandra Złotkowska 3, Robert Milewski 4





# RNA integrity and differential expression

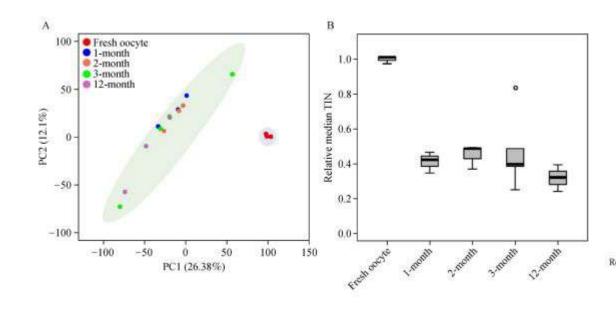
> Front Med, 2021 Feb;15(1):144-154. doi: 10.1007/s11684-020-0792-7. Epub 2020 Sep 2.

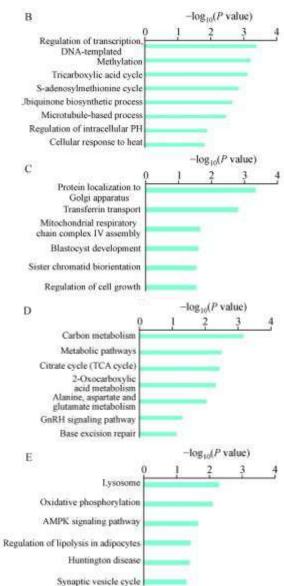
# Effects of vitrification and cryostorage duration on single-cell RNA-Seq profiling of vitrified-thawed human metaphase II oocytes

```
Ying Huo * ^{1} ^{2} ^{3} ^{4}, Peng Yuan * ^{1} ^{3} ^{4}, Qingyuan Qin * ^{1} ^{3} ^{4}, Zhiqiang Yan ^{1} ^{3} ^{4} ^{5}, Liying Yan ^{1} ^{3} ^{4} ^{6}, Ping Liu ^{1} ^{3} ^{4} ^{6}, Rong Li ^{1} ^{3} ^{4} ^{6}, Jie Yan ^{7} ^{8} ^{9}, Jie Qiao ^{1} ^{3} ^{4} ^{5} ^{6}
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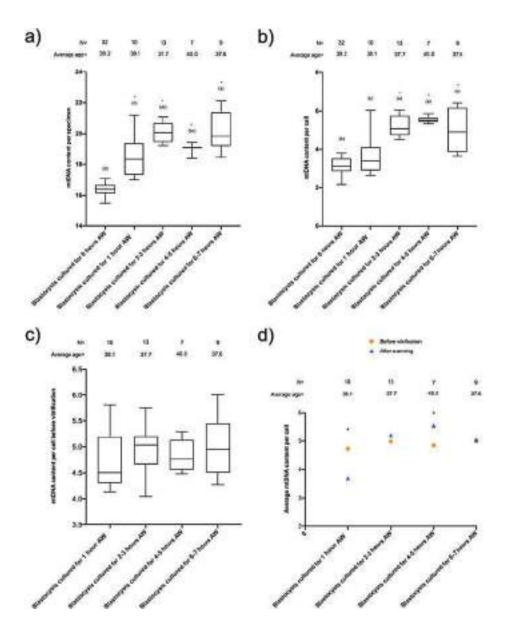
Affiliations + expand

PMID: 32876878 DOI: 10.1007/s11684-020-0792-7





- The RNA integrity in vitrified oocytes was lower compared to fresh oocytes.
- No difference was observed between vitrified oocytes in RNA integrity during the 1<sup>st</sup> year of cryopreservation
- A number of DEGs were observed leading to different biological processes



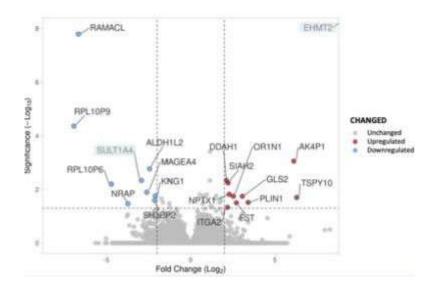
It may seem to be better to leave blastocysts for 6 hours prior to ET

### The effect of vitrification on blastocyst mitochondrial DNA dynamics and gene expression profiles

Marta Pérez Sánchez <sup>1</sup>, Maria Luisa Pardiñas <sup>1</sup>, Antonio Diez Juan <sup>2</sup>, Alicia Quiñonero <sup>1</sup>, Francisco Dominguez <sup>1</sup>, Angel Martin <sup>1</sup>, Carmina Vidal <sup>3</sup>, Diana Beltrán <sup>4</sup>, Amparo Millaud <sup>4</sup>, Amparo Mercades <sup>4</sup>, Amonio Pellicer <sup>5</sup>, Ana Cobo <sup>4</sup>, Maria José de los Santos <sup>4,85</sup>

mtDNA content of the blastocyst is lower during the first hour post-thaw, then increases sharply and seems to be at pre-vitrification levels at about **6-hours post thaw**.

Over 100 DEGs were observed between 0h post-thaw and 4-5 hours post-thaw, affecting 186 biological processes.



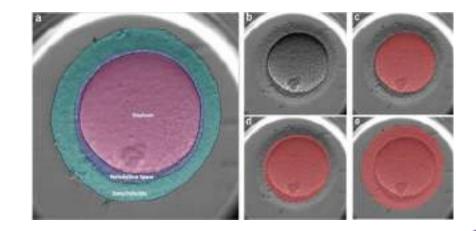
# Summary II

An extended post-thaw time (around 6h) may be beneficial for blastocysts.

Blastocyst vitrification seems to lead to improved results when performed on D5 versus D6 (diff KPIs?)

Vitrification seems to result to differential gene expression both in oocytes and in embryos (FU)

# What about Al



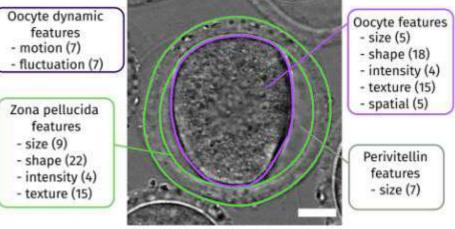
- Morphology may not be uninformative but the way we evaluate it may be.
- Al may provide a new perspective.
- Should we consider AI to be introduced in KPIs?
- No before it is tested and validated.

Article Open access Published: 08 May 2024

### Segmentation of mature human oocytes provides interpretable and improved blastocyst outcome predictions by a machine learning model

Julin Fieldstad [2], Weikai Qi, Nadia Siddique, Natalie Mercuri Dan Navot & Alex Krivoi

Scientific Reports 14, Article number 10569 (2024) | Cite this article



© 2522. Published by The Company of Biologists Ltd | Journal of Cell Science (2022) 135, jos260281, doi:10.12425cs.286261



#### TOOLS AND RESOURCES

An interpretable and versatile machine learning approach for oocyte phenotyping

Gaelle Letort<sup>1,\*</sup>, Adrien Eichmuller<sup>1</sup>, Christelle Da Silva<sup>1</sup>, Elvira Nikalayevich<sup>1</sup>, Flora Crozet<sup>1</sup>, Jeremy Salle<sup>2</sup>, Nicolas Minc<sup>2</sup>, Elsa Labrune<sup>3,4,5</sup>, Jean-Philippe Wolf<sup>6,7</sup>, Marie-Emilie Terret<sup>1</sup> and Marie-Hélène Verlhac<sup>1</sup>

# How long is it safe to cryopreserve an embryo? Different KPIs for different cryo times?

5 Seprod Biomed Online, 2023 Oct.47/6;103254, doi: 10.7016/j.dumo.3023.06.089. Epub 2023 Jun 23.

"Open Debate"

Storage time does not influence pregnancy and neonatal outcomes for first single vitrified highquality blastocyst transfer cycle

Yuanlin Ma ., Mingra Sun ., Tianna Wen ., Chenhai Ding .. Lok-Wan Lio .. Tian Meng .. runi Song 1 Xuerong Frou 1 Gingyun Mai 1 Tomwen Xu 2

> 3/ Reprint Blamed Online, 2018 Jun 36(4):614-611, dot 10.1016/j./tmo.2018.03.008. Coup 2010 Nac 14.

Cryostorage duration does not affect pregnancy and neonatal outcomes: a retrospective single-centre cohort study of vitrified-warmed blastocysts

Sarpa Ni Idanui 1, Kazun Didhiyama 1, Tomoko Kuroda 5, Akiko Yabaumi 1, Keriji Emis 1, Technic Chimura 1 Indonti Dicoro 2 Semenu Katuranto 1 Krischi Eate 2

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The effect of extended cryo-storage following: vitrification on embryo competence: a systematic review and meta-analysis

A DANGER T, If Committee T, A control T, If thought T, A house M A trade T, Frank T, 3) Rocco T. A. Welstell, T. G. Samurell, T. A. Breeff, T. B. Stephanel, T. C. Sanger, P. H. Sharle, T.

# Engagerian (January) 2007 Dec 25:12:734063 doi: 10.1009/endo2021.724053

The Impact of Embryo Storage Time on Pregnancy and Perinatal Outcomes and the Time Limit of Vitrification: A Retrospective Cohort Study

> mr J Gynesool Obnier, 2021 Mar 152 (1):351-357, doi: 10.1002/figs. Mengge Cal.<sup>1</sup>, Jupan Dong.<sup>1</sup>, Shahar Lou<sup>2</sup>, Yu Zheng.<sup>1</sup>, Jingi R.<sup>2</sup>

Does duration of cryostorage affect survival rate, pregnancy, and neonatal outcomes? Large-scale single-center study of slush nitrogen (SN2) vitrified-warmed blastocysts

Hor Nam Lat. Jan Com Park. Soc Syang Park. A Houn Boan. Havegook Sping. Her Jun Lee 1, Eur Mi Chang 1, & Won Kire 1, Woo Sik Lee 1, Sang Woo Lyu 1

+ Foort Entrodiest (Laurence) 2621 Sept 27;12:209548, doi: 11.1399/femin.2001.09948/3

Prolonged Cryopreservation Negatively Affects Embryo Transfer Outcomes Following the Elective Freeze-All Strategy: A Multicenter Retrospective Study

Audona Zhune 12", Skombon wu LT, Gurernitten 1, Ruspins Wu 1, Paucie Ber 2", Virlanz Zhune 1, Armin Yone "Alterna B." Lina Bal." Acrahan Zhone " Adrum Ing 14"

The effect of storage time after vitrification on pregnancy and neonatal outcomes among 24 698 patients following the first embryo transfer cycles

Harghal LL Mingra NV, Blan Wong, Having Lin, Okias Chen, Ningling Young Office Lon. Yor Warry, Sampley Favory #1, Champion Tho #2 Author Sales

Warmen Augmodication, reducted bis, home 7, adaptation, Pages 2615-3468. Piteto/Vincep/18.1955/Surregodess/18-

2 Famil StariL 2023 Janet 19(1):36-44. doi: 10.1016/j.formstart.2022.10.016. Epob 2022 Nov 20.

Pregnancy and neonatal outcomes after long-term vitrification of blastocysts among 6,900 patients after their last live birth

Yueyue Yan 1. Qian Zhang 1, Linkn Yang 1, Wei Zhou 1, Hanxang Ni 1, Junhao Yan 2

> Fertil Steril, 2022 Sep;113(3):513-521. doi:10.1016().fertiluteri.2022.06.003. Epub 2022 Aug 5.

The association between embryo storage time and treatment success in women undergoing freeze-all embryo transfer

Kei-Lustin \* Sereh Hunt \* Dan Zhang \* Song 11 \* Ben W Mol \*

Britis Analysis 3 J Avres Reprint Genet, 2004 Dc041(16):2681-3786. doi:10.1017/s19815-624-12361-7-5pub.2884-3ct-16

Impact of prolonged embryo storage on reproductive and neonatal outcomes: a systematic review and meta-analysis

here 2010/08.7, Monta Noneu villerina 7, para bestit beralla dacca 7, Incellation forcio flution? Los blooms Minales?

# Take-home messages

With vitrification on the rise, It is high time to revisit KPIs

In the era of precision medicine and artificial intelligence KPIs can no longer be based on morphology and clinical outcomes

Addition of molecular data and AI could be considered in the future. Further interdisciplinary research on molecular effects and time.

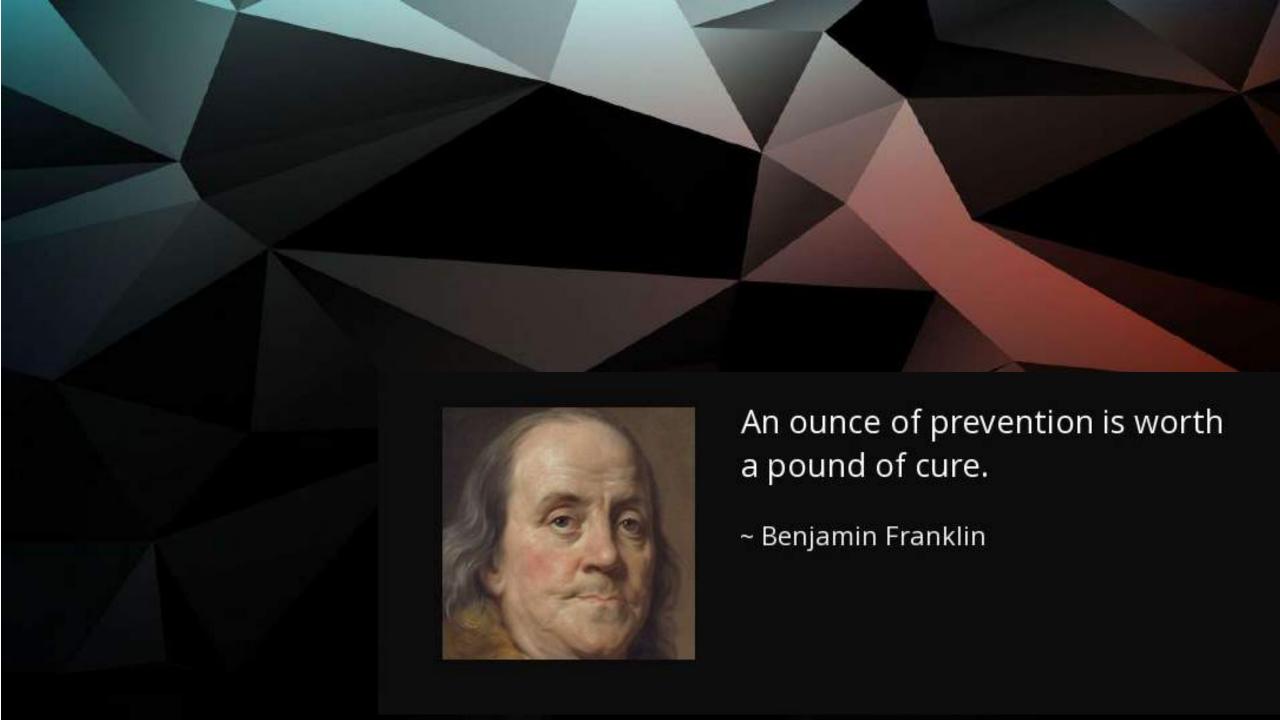
Clinical outcomes are important; however, the molecular content may affect the health status of the neonate in the long-term.

# Is the research that we do enough?



- **Multi-disciplinary**: people from different disciplines working together, each drawing on their disciplinary knowledge.
- **Cross-disciplinary**: viewing one discipline from the perspective of another.
- Inter-disciplinary: integrating knowledge and methods from different disciplines, using a real synthesis of approaches

<u>Interdisciplinary Approach</u> ... Medical Doctor, Clinical Embryologist, Experimental Biologist, Computational Biologist, Product Developer, Marketing, Software Developer, Surgical Robotics, Biomedical Engineer, Computer Vision Engineer, Robotics Engineer, Mechatronics Engineer...







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#### Advanced Course

Clinical Embryology and Physiology of Preimplantation Embryo



#### Programme of Teaching Module 6

"Understanding the Embryo and Culture Systems: Mapping of state-of-the-art Equipment and Protocols"

Submodule	Lecture-Speakers	Date-Time
6.1	Physiology and development of the preimplantation embryo Mara Simopoulou	2/3/2022 6:00 p.m - 7:00 p.m
6.2	The pyramid of successful implantation  Lowro Rienzi	2/3/2022 7:00 p.m - 8:00 p.m
6.3	Metabolism of the pre-mplantation surbryo Roger Starmey	9/3/2022 5:00 p.m - 6:00 p.m
6.4	Culture media in IVF Deun Morbeck	9/3/2022 6:00 p.m – 7:00 p.m
6.5	Embryo culture incabators Jason E. Swain	16/3/2022 5:00 p.m - 6:00 p.m
6.6	Time-lapse imaging technology in the IVF laboratory Morcos Meseguer	16/3/2022 6:00 p.m - 7:00 p.m
6.7	Troubleshooting in embryo adture Eros Nikitos	23/2/2022 5:00 p.m - 6:00 p.m
6.8	Embryo culture and Evidence Based Clinical Practice David Gardner	30/3/2022 8:00 am - 9:00 a -
		THE RESIDENCE OF THE PARTY OF T



# Thank You!



**Contact details** 

Email: marasimopoulou@hotmail.com



