HYGEIA IVF EMBRYOGENESIS

Optimizing vitrification and warming protocols

Ioannis Sfontouris M.Med.Sci, PhD

Director of Embryology Laboratory - Hygeia IVF Embryogenesis Athens

Clinical Associate Professor – University of Nicosia

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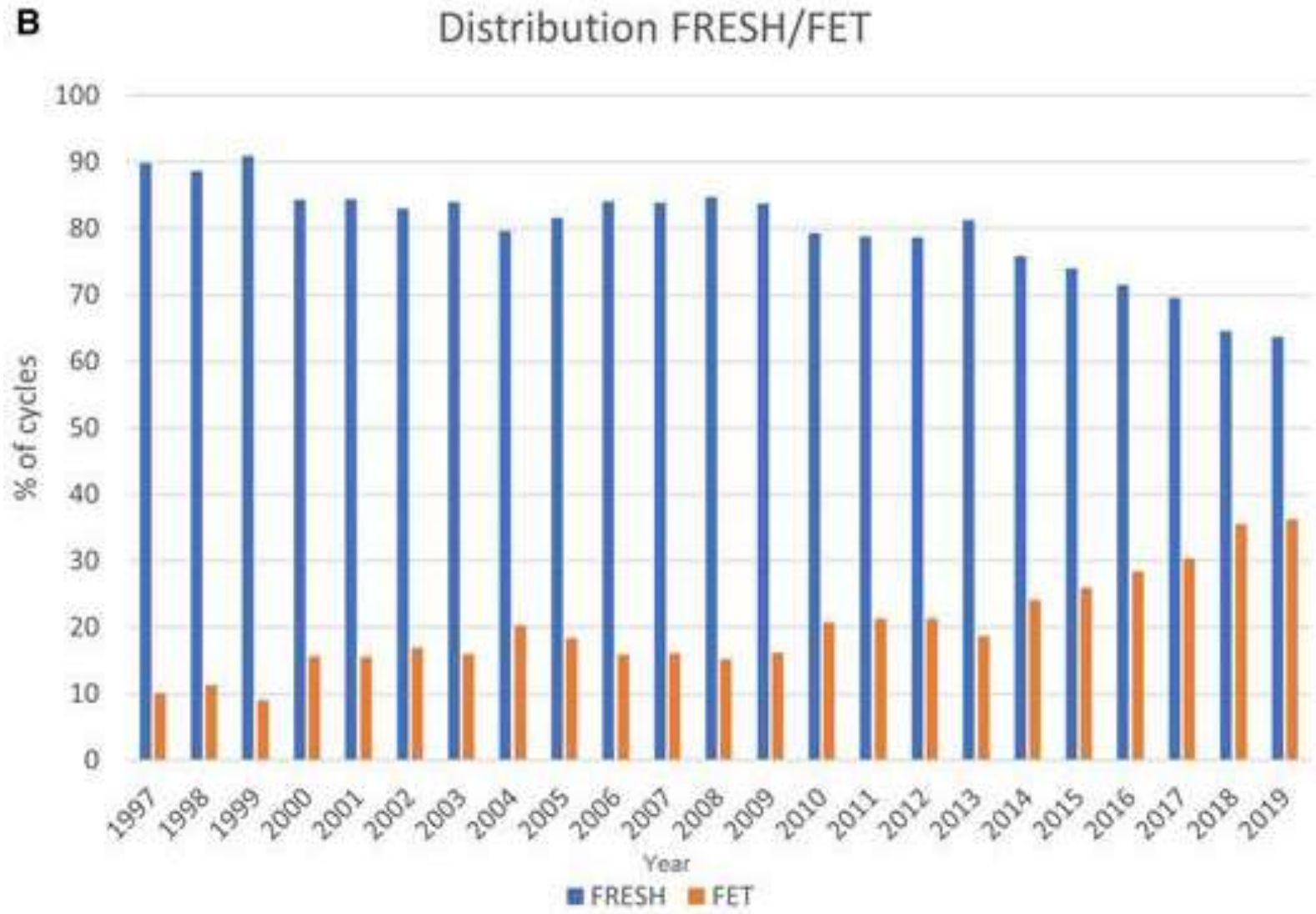
Hotel Royal Continental

Progressive increase in the use of FET relative to fresh IVF/ICSI cycles in Europe (EIM 2019 data)



Smeenk et al 2023 HR

Cryopreservation is a game changer in ART

- Oocyte freezing for social and medical reasons
- Supernumerary embryos
- PGT cycles
- Prevention of OHSS
- Elective single embryo transfer (eSET)
- High PRG – suboptimal endometrium
- Elective freeze-all

Human Reproduction Update, Vol.23, No.2 pp. 139–155, 2017
Advanced Access publication on November 1, 2016 doi:10.1093/humupd/dmw038

human reproduction update

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

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

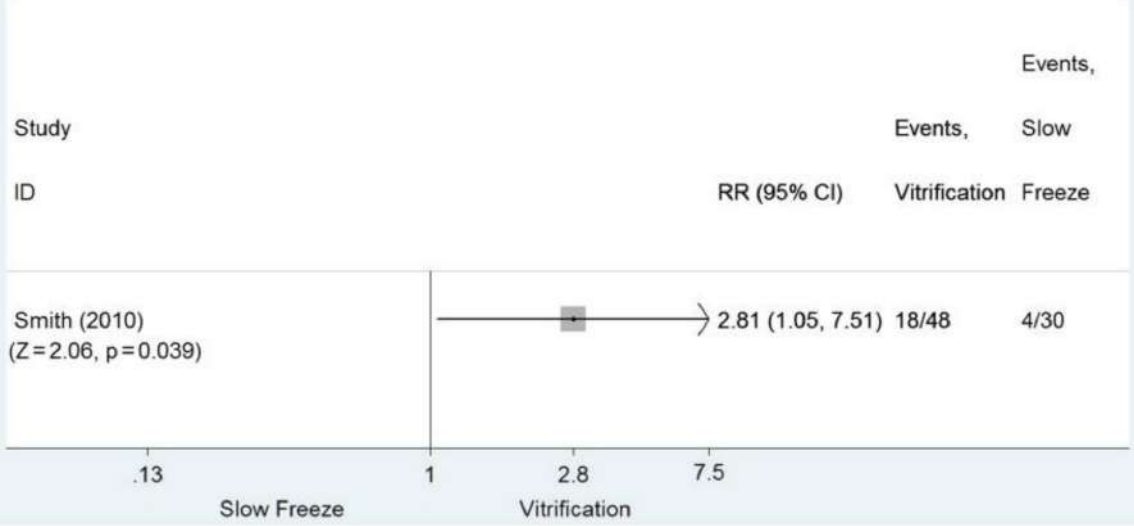
- **Higher CPR** embryo vitrification compared with slow-freezing, (RR = 1.89, 95% CI: 1.00–3.59; $P = 0.051$; three RCTs; $I^2 = 71.9\%$). 3 RCTs

- **Higher LBR** for embryo vitrification compared to slow-freezing (RR = 2.28; 95% CI: 1.17–4.44; $P = 0.016$; 216 cycles; one RCT).

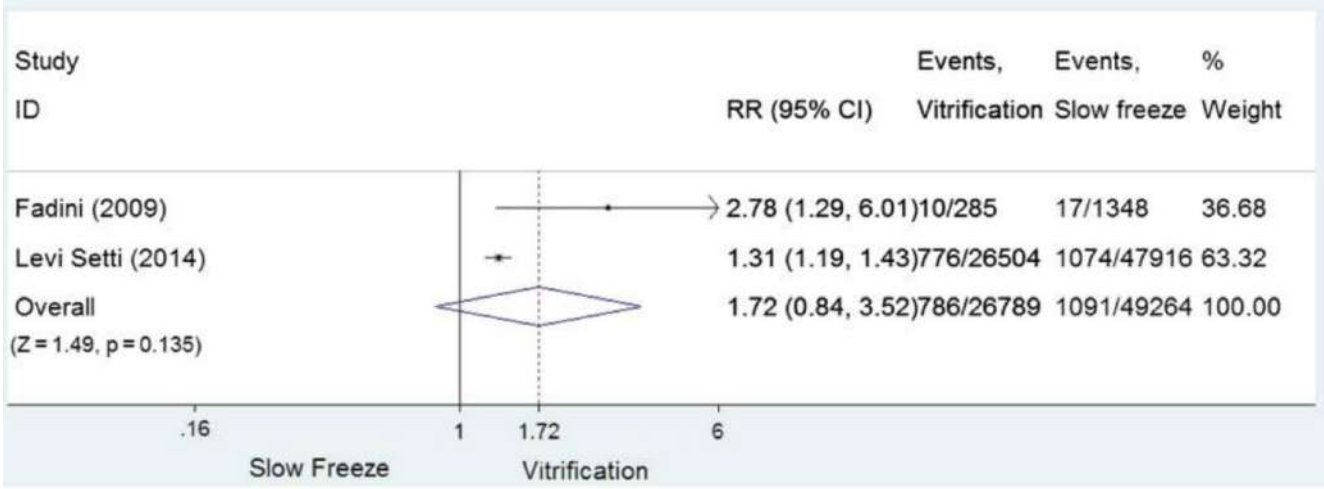
- **Better embryo cryosurvival** with vitrification compared with slow-freezing (RR = 1.59, 95% CI: 1.30–1.93; $P < 0.001$; $I^2 = 93\%$). 7 RCTs

Oocyte cryopreservation: vitrification vs slow

Higher ongoing pregnancy rate per cycle using vitrification RR = 2.81, 95% CI: 1.05–7.51; *P* = 0.039



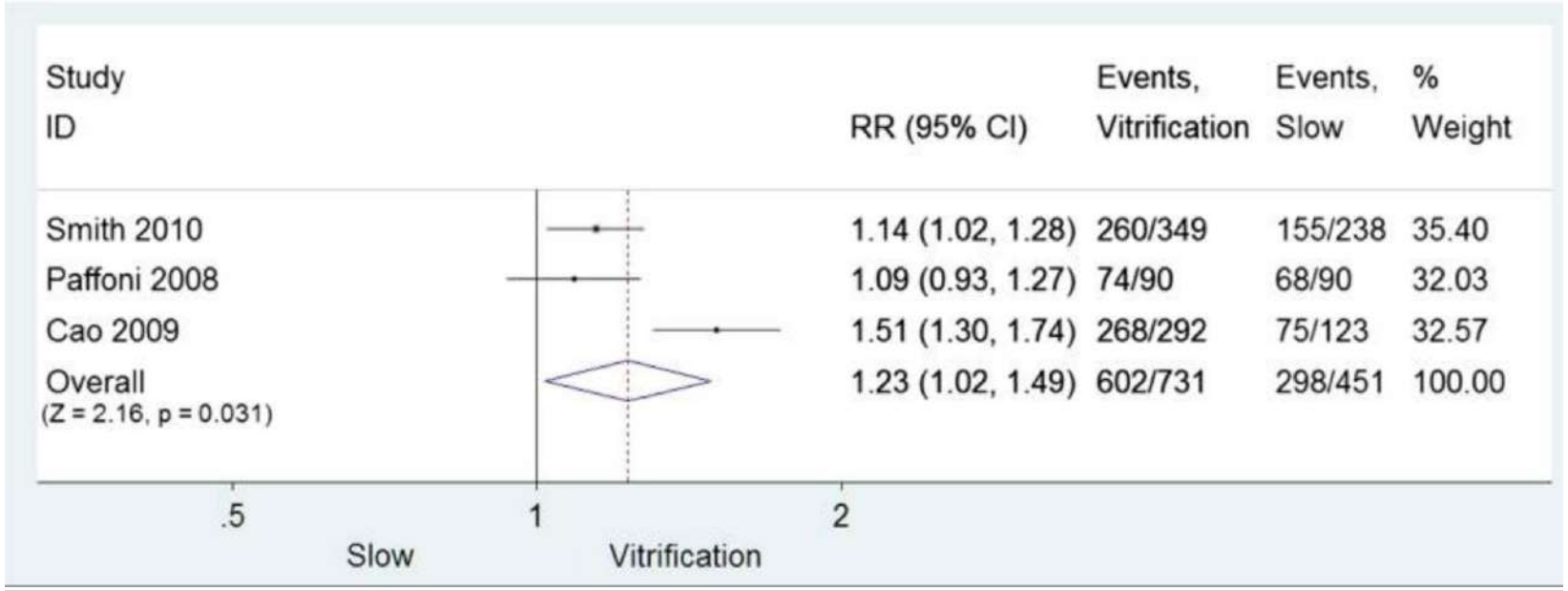
Higher ongoing pregnancy rate per oocyte warmed using vitrification RR = 1.14, 95% CI: 1.02–1.28; *P* = 0.018



Rienzi et al 2017 HRU

Oocyte cryopreservation: vitrification vs slow

Better cryo-survival using vitrification (83.2%) vs slow freezing (66.1%)



Rienzi et al 2017 HRU

Techniques used in embryo cryopreservation

Vitrification vs slow freezing	Blastocyst collapse	Type of vitrification carrier (open or closed)	Loading volume	Post-thaw culture prior to transfer
Semi-automated vitrification	Composition of cryoprotectant solutions	Cleavage-stage vs blastocyst	Degree of blastocyst expansion	Assisted hatching post-thaw

Cochrane review in preparation

Sfontouris IA, Makris A, Barbosa MWP, Storr A, Raine-Fenning N,

Hart RJ, Venetis C, Martins WP.

Techniques for human embryo cryopreservation

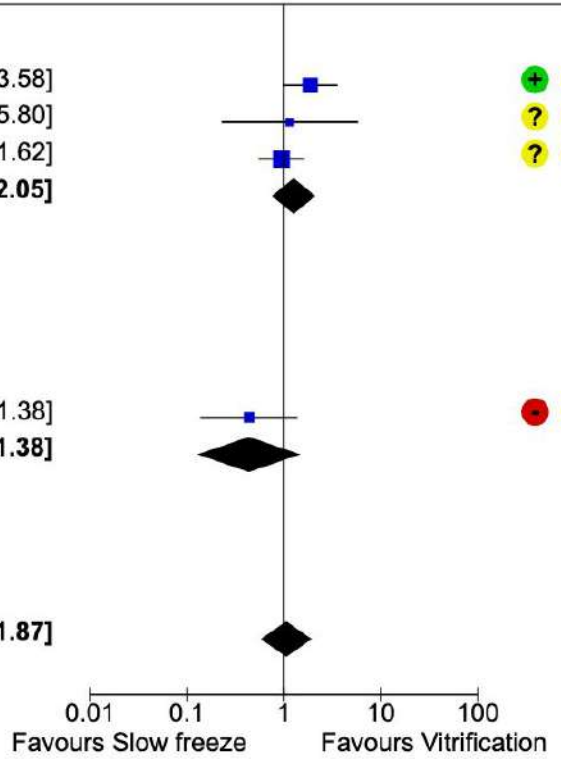
Cochrane Database of Systematic Reviews, CD009589

In preparation

- 37 RCTs
- 19 comparisons were identified in published RCTs

Slow freezing vs vitrification - Live birth

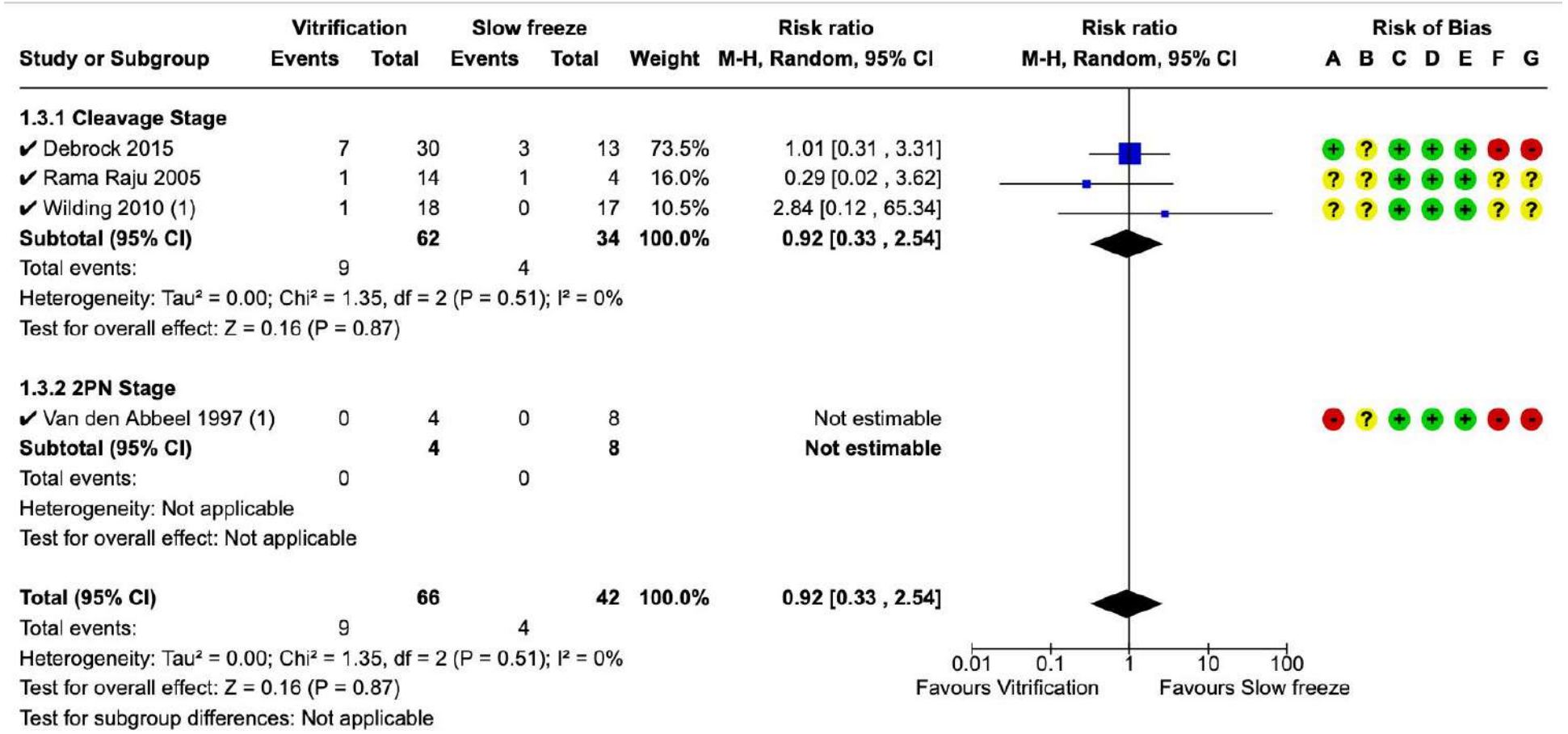
Study or Subgroup	Vitrification Events	Vitrification Total	Slow freeze Events	Slow freeze Total	Weight	Risk ratio M-H, Random, 95% CI	Risk of Bias A B C D E F G
1.1.1 Cleavage stage							
✔ Debrock 2015	29	109	10	70	33.6%	1.86 [0.97 , 3.58]	+ ? + + + - -
✔ Rama Raju 2005	4	40	2	23	10.2%	1.15 [0.23 , 5.80]	? ? + + + ? ?
✔ Wilding 2010	17	51	17	48	38.9%	0.94 [0.55 , 1.62]	? ? + + + ? ?
Subtotal (95% CI)		**200**		**141**	**82.7%**	**1.26 [0.77 , 2.05]**	
Total events:	50		29				
Heterogeneity: Tau² = 0.04; Chi² = 2.55, df = 2 (P = 0.28); I² = 21%							
Test for overall effect: Z = 0.93 (P = 0.35)							
1.1.2 2PN Stage							
✔ Van den Abbeel 1997 (1)	4	78	8	68	17.3%	0.44 [0.14 , 1.38]	- ? + + + - -
Subtotal (95% CI)		**78**		**68**	**17.3%**	**0.44 [0.14 , 1.38]**	
Total events:	4		8				
Heterogeneity: Not applicable							
Test for overall effect: Z = 1.41 (P = 0.16)							
Total (95% CI)		**278**		**209**	**100.0%**	**1.06 [0.60 , 1.87]**	
Total events:	54		37				
Heterogeneity: Tau² = 0.14; Chi² = 5.29, df = 3 (P = 0.15); I² = 43%							
Test for overall effect: Z = 0.19 (P = 0.85)							
Test for subgroup differences: Chi² = 2.75, df = 1 (P = 0.10), I² = 63.6%							



- Similar LBR with slow freezing vs vitrification
- No RCT reporting LBR to compare slow vs vitrification of blastocysts

Sfontouris et al, Unpublished

Slow freezing vs vitrification - Miscarriage



Study or Subgroup	Vitrification Events	Vitrification Total	Slow freeze Events	Slow freeze Total	Weight	Risk ratio M-H, Random, 95% CI
1.3.1 Cleavage Stage						
✔ Debrock 2015	7	30	3	13	73.5%	1.01 [0.31 , 3.31]
✔ Rama Raju 2005	1	14	1	4	16.0%	0.29 [0.02 , 3.62]
✔ Wilding 2010 (1)	1	18	0	17	10.5%	2.84 [0.12 , 65.34]
Subtotal (95% CI)		**62**		**34**	**100.0%**	**0.92 [0.33 , 2.54]**
Total events:	9		4			
Heterogeneity: Tau² = 0.00; Chi² = 1.35, df = 2 (P = 0.51); I² = 0%						
Test for overall effect: Z = 0.16 (P = 0.87)						
1.3.2 2PN Stage						
✔ Van den Abbeel 1997 (1)	0	4	0	8		Not estimable
Subtotal (95% CI)		**4**		**8**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
Total (95% CI)		**66**		**42**	**100.0%**	**0.92 [0.33 , 2.54]**
Total events:	9		4			
Heterogeneity: Tau² = 0.00; Chi² = 1.35, df = 2 (P = 0.51); I² = 0%						
Test for overall effect: Z = 0.16 (P = 0.87)						
Test for subgroup differences: Not applicable						

- Similar miscarriage rate with slow freezing vs vitrification

Sfontouris et al, Unpublished

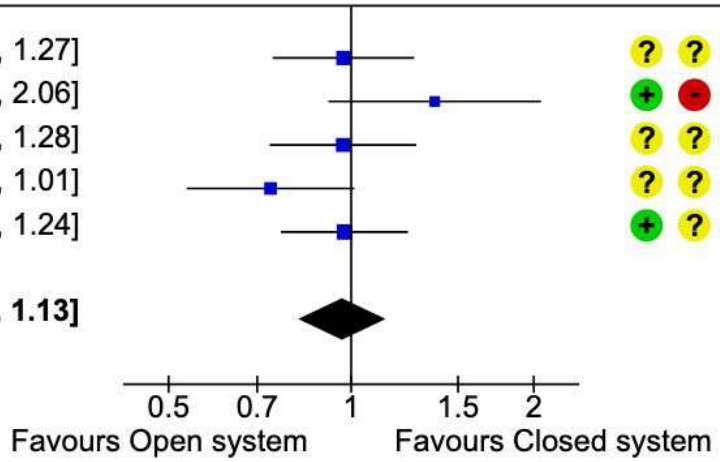
Open vs closed vitrification – Live birth

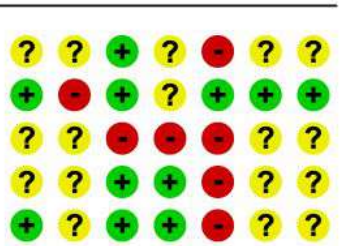
Study or Subgroup	Closed system Events	Total	Open system Events	Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Amo 2013	48	135	119	325	22.6%	0.97 [0.74 , 1.27]
✔ Balaban 2007 (1)	39	101	27	96	12.3%	1.37 [0.92 , 2.06]
✔ Hashimoto 2013	44	100	74	163	21.5%	0.97 [0.73 , 1.28]
✔ Kim 2017	39	107	50	101	17.8%	0.74 [0.54 , 1.01]
✔ Panagiotidis 2013	84	224	80	208	25.9%	0.97 [0.77 , 1.24]
Total (95% CI)		**667**		**893**	**100.0%**	**0.97 [0.83 , 1.13]**
Total events:	254		350			

Heterogeneity: Tau² = 0.01; Chi² = 5.73, df = 4 (P = 0.22); I² = 30%

Test for overall effect: Z = 0.44 (P = 0.66)

Test for subgroup differences: Not applicable





- Similar LBR with open and closed vitrification carriers

Sfontouris et al, Unpublished

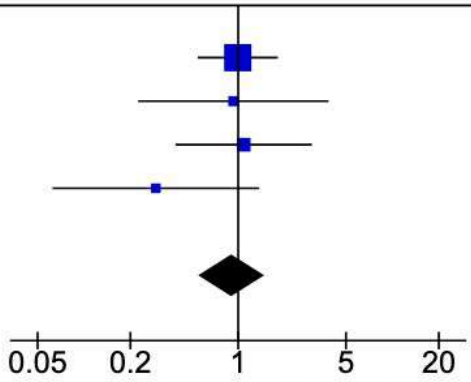
Open vs closed vitrification - Miscarriage

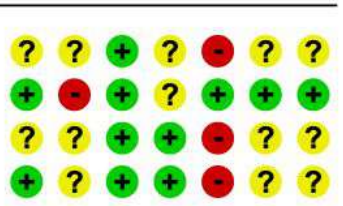
Study or Subgroup	Closed system Events	Total	Open system Events	Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Amo 2013 (1)	12	60	30	149	59.8%	0.99 [0.55 , 1.81]
✔ Balaban 2007	4	43	3	30	10.6%	0.93 [0.22 , 3.86]
✔ Kim 2017 (2)	6	45	7	57	20.7%	1.09 [0.39 , 3.00]
✔ Panagiotidis 2013	2	87	7	89	9.0%	0.29 [0.06 , 1.37]
Total (95% CI)		**235**		**325**	**100.0%**	**0.90 [0.57 , 1.43]**
Total events:	24		47			

Heterogeneity: Tau² = 0.00; Chi² = 2.31, df = 3 (P = 0.51); I² = 0%

Test for overall effect: Z = 0.45 (P = 0.66)

Test for subgroup differences: Not applicable





- Similar miscarriage with open and closed vitrification carriers

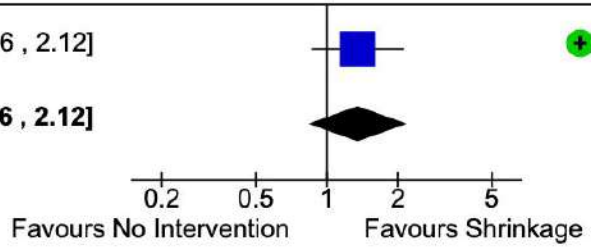
Sfontouris et al, Unpublished

Artificial blastocyst shrinkage vs no intervention

Live birth

Study or Subgroup	Shrinkage Events	Shrinkage Total	No intervention Events	No intervention Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Gala 2014	23	67	30	118	100.0%	1.35 [0.86 , 2.12]
Total (95% CI)		**67**		**118**	**100.0%**	**1.35 [0.86 , 2.12]**
Total events:	23		30			

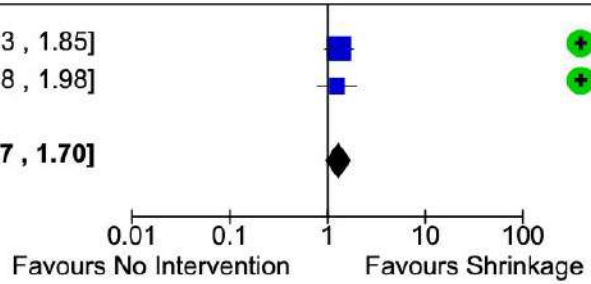
Heterogeneity: Not applicable
Test for overall effect: Z = 1.30 (P = 0.19)
Test for subgroup differences: Not applicable



Clinical pregnancy

Study or Subgroup	Shrinkage Events	Shrinkage Total	No intervention Events	No intervention Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Gala 2014	32	67	43	118	64.7%	1.31 [0.93 , 1.85]
✔ Van Landuyt 2015	26	69	21	69	35.3%	1.24 [0.78 , 1.98]
Total (95% CI)		**136**		**187**	**100.0%**	**1.28 [0.97 , 1.70]**
Total events:	58		64			

Heterogeneity: Tau² = 0.00; Chi² = 0.04, df = 1 (P = 0.85); I² = 0%
Test for overall effect: Z = 1.76 (P = 0.08)
Test for subgroup differences: Not applicable



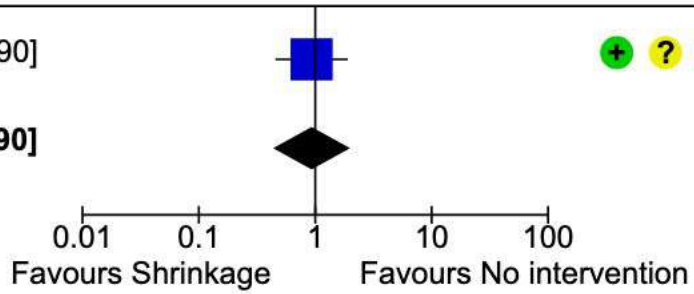
- Higher CPR but similar LBR with blastocyst shrinkage

Sfontouris et al, Unpublished

Artificial blastocyst shrinkage vs no intervention Miscarriage

Study or Subgroup	Shrinkage Events	Shrinkage Total	No intervention Events	No intervention Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Gala 2014 (1)	9	32	13	43	100.0%	0.93 [0.45 , 1.90]
Total (95% CI)		**32**		**43**	**100.0%**	**0.93 [0.45 , 1.90]**
Total events:	9		13			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.20 (P = 0.84)
Test for subgroup differences: Not applicable



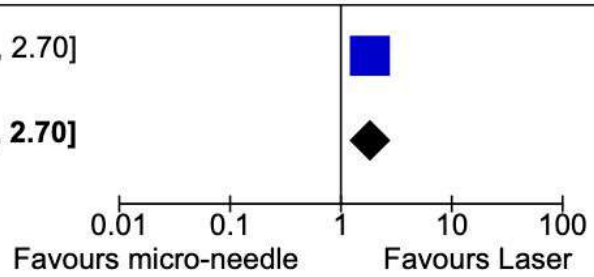
- Similar miscarriage with blastocyst shrinkage vs no intervention

Sfontouris et al, Unpublished

Laser vs mechanical blastocyst shrinkage Clinical pregnancy

Study or Subgroup	Laser Pulse Events	Laser Pulse Total	Micro-needle Events	Micro-needle Total	Weight	Odds ratio M-H, Random, 95% CI	Odds ratio M-H, Random, 95% CI	Risk of Bias A	B	C	D	E	F	G
✔ Kamel Mohamed 2021	123	205	92	204	100.0%	1.83 [1.23 , 2.70]		?	?	+	+		?	?
Total (95% CI)		**205**		**204**	**100.0%**	**1.83 [1.23 , 2.70]**								
Total events:	123		92											

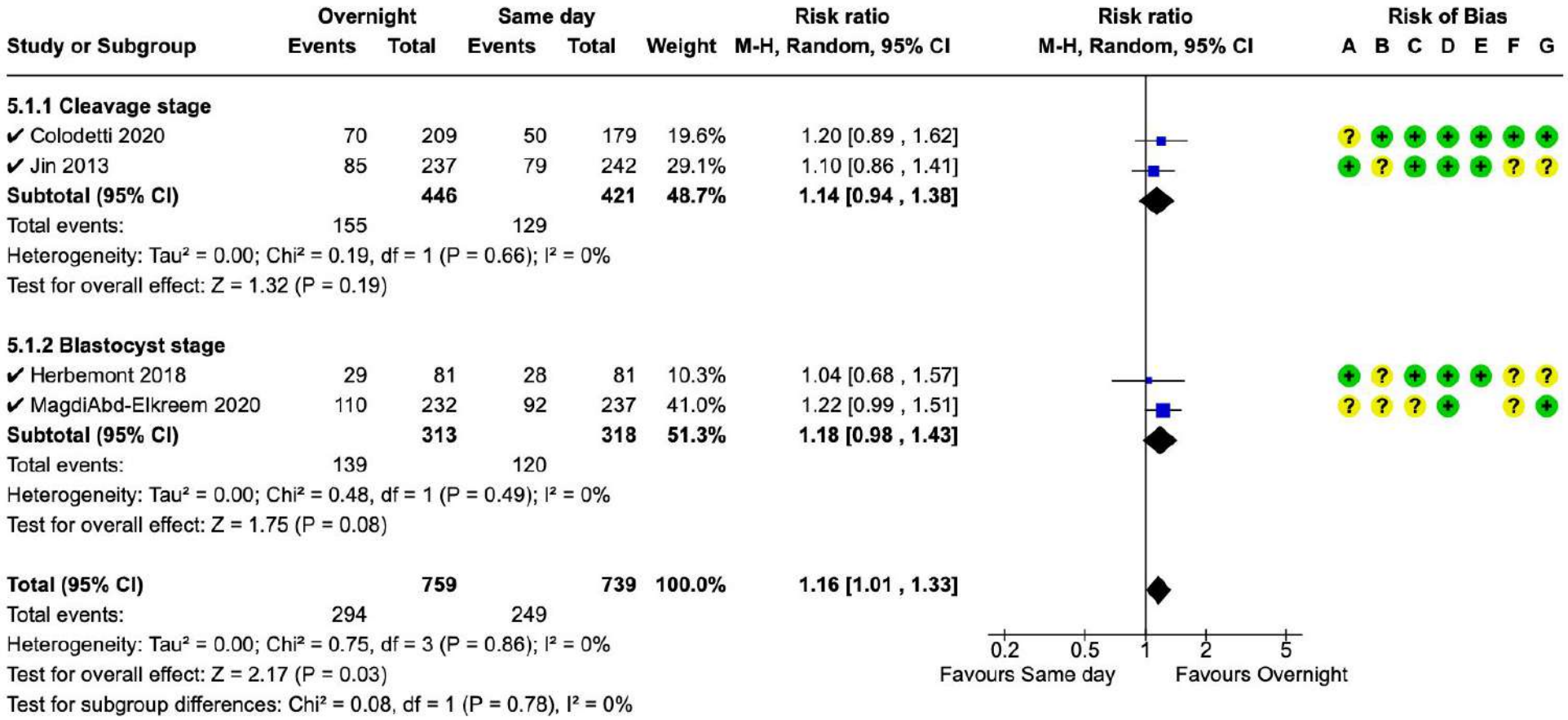
Heterogeneity: Not applicable
Test for overall effect: Z = 3.01 (P = 0.003)
Test for subgroup differences: Not applicable



- Higher CPR using laser shrinkage vs mechanical
- Data on Live birth and miscarriage not reported in any RCT

Sfontouris et al, Unpublished

FET same day vs overnight culture – Live birth

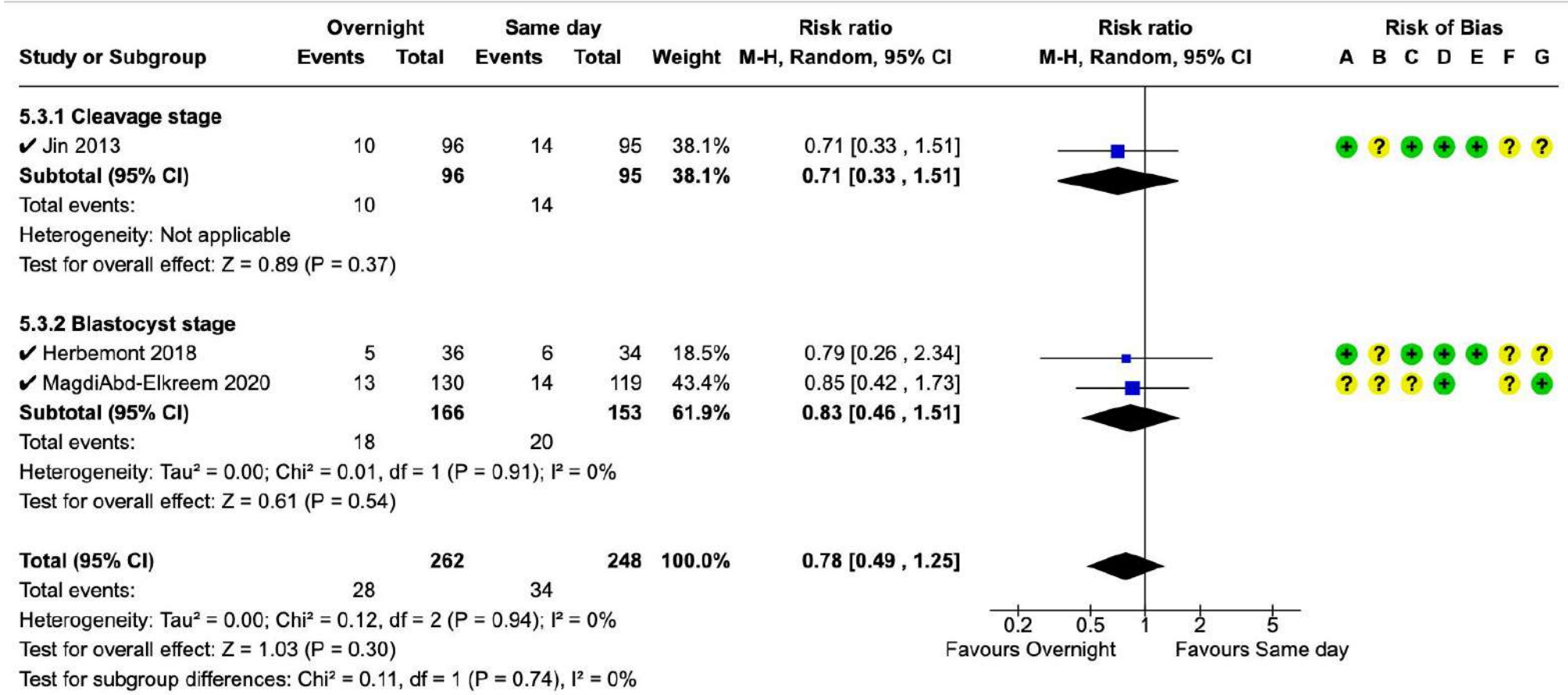


Study or Subgroup	Overnight Events	Total	Same day Events	Total	Weight	Risk ratio M-H, Random, 95% CI
5.1.1 Cleavage stage						
✔ Colodetti 2020	70	209	50	179	19.6%	1.20 [0.89 , 1.62]
✔ Jin 2013	85	237	79	242	29.1%	1.10 [0.86 , 1.41]
Subtotal (95% CI)		**446**		**421**	**48.7%**	**1.14 [0.94 , 1.38]**
Total events:	155		129			
Heterogeneity: Tau² = 0.00; Chi² = 0.19, df = 1 (P = 0.66); I² = 0%						
Test for overall effect: Z = 1.32 (P = 0.19)						
5.1.2 Blastocyst stage						
✔ Herbemont 2018	29	81	28	81	10.3%	1.04 [0.68 , 1.57]
✔ MagdiAbd-Elkreem 2020	110	232	92	237	41.0%	1.22 [0.99 , 1.51]
Subtotal (95% CI)		**313**		**318**	**51.3%**	**1.18 [0.98 , 1.43]**
Total events:	139		120			
Heterogeneity: Tau² = 0.00; Chi² = 0.48, df = 1 (P = 0.49); I² = 0%						
Test for overall effect: Z = 1.75 (P = 0.08)						
Total (95% CI)		**759**		**739**	**100.0%**	**1.16 [1.01 , 1.33]**
Total events:	294		249			
Heterogeneity: Tau² = 0.00; Chi² = 0.75, df = 3 (P = 0.86); I² = 0%						
Test for overall effect: Z = 2.17 (P = 0.03)						
Test for subgroup differences: Chi² = 0.08, df = 1 (P = 0.78), I² = 0%						

- Higher LBR with overnight culture

Sfontouris et al, Unpublished

FET same day vs overnight culture – Miscarriage



Study or Subgroup	Overnight Events	Overnight Total	Same day Events	Same day Total	Weight	Risk ratio M-H, Random, 95% CI
5.3.1 Cleavage stage						
✔ Jin 2013	10	96	14	95	38.1%	0.71 [0.33 , 1.51]
Subtotal (95% CI)		**96**		**95**	**38.1%**	**0.71 [0.33 , 1.51]**
Total events:	10		14			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.89 (P = 0.37)						
5.3.2 Blastocyst stage						
✔ Herbemont 2018	5	36	6	34	18.5%	0.79 [0.26 , 2.34]
✔ MagdiAbd-Elkreem 2020	13	130	14	119	43.4%	0.85 [0.42 , 1.73]
Subtotal (95% CI)		**166**		**153**	**61.9%**	**0.83 [0.46 , 1.51]**
Total events:	18		20			
Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 1 (P = 0.91); I² = 0%						
Test for overall effect: Z = 0.61 (P = 0.54)						
Total (95% CI)		**262**		**248**	**100.0%**	**0.78 [0.49 , 1.25]**
Total events:	28		34			
Heterogeneity: Tau² = 0.00; Chi² = 0.12, df = 2 (P = 0.94); I² = 0%						
Test for overall effect: Z = 1.03 (P = 0.30)						
Test for subgroup differences: Chi² = 0.11, df = 1 (P = 0.74), I² = 0%						

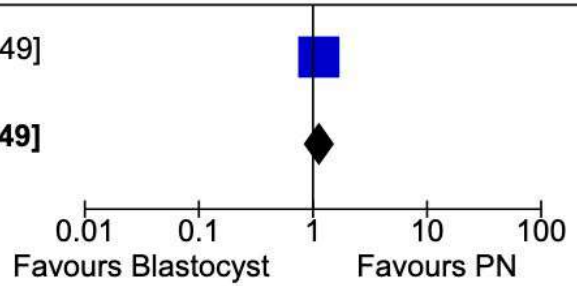
- Similar miscarriage rate

Sfontouris et al, Unpublished

Pronuclear vs blastocyst vitrification
Live birth

Study or Subgroup	PN Events	PN Total	Blastocyst Events	Blastocyst Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Shapiro 2015	44	71	38	69	100.0%	1.13 [0.85 , 1.49]
Total (95% CI)		**71**		**69**	**100.0%**	**1.13 [0.85 , 1.49]**
Total events:	44		38			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.83 (P = 0.41)
Test for subgroup differences: Not applicable



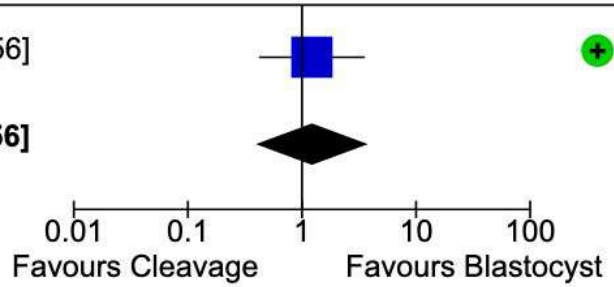
- Similar LBR

Sfontouris et al, Unpublished

Pronuclear vs blastocyst vitrification Miscarriage

Study or Subgroup	Cleavage Events	Cleavage Total	Blastocyst Events	Blastocyst Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Shapiro 2015	7	47	5	41	100.0%	1.22 [0.42 , 3.56]
Total (95% CI)		**47**		**41**	**100.0%**	**1.22 [0.42 , 3.56]**
Total events:	7		5			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.37 (P = 0.71)
Test for subgroup differences: Not applicable



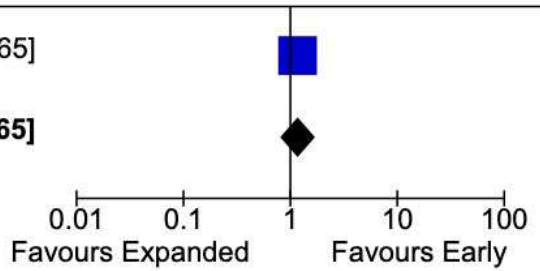
- Similar miscarriage rate

Sfontouris et al, Unpublished

Early vs expanded blastocyst vitrification
Live birth

Study or Subgroup	Early Blastocysts Events	Total	Expanded Blastocysts Events	Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Rama Raju 2009	68	281	40	193	100.0%	1.17 [0.83 , 1.65]
Total (95% CI)		**281**		**193**	**100.0%**	**1.17 [0.83 , 1.65]**
Total events:	68		40			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.88 (P = 0.38)
Test for subgroup differences: Not applicable



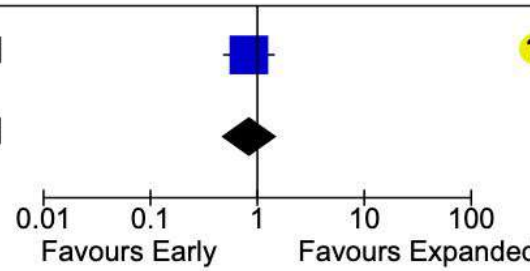
- Similar LBR

Sfontouris et al, Unpublished

Early vs expanded blastocyst vitrification Miscarriage

Study or Subgroup	Early Blastocysts Events	Total	Expanded Blastocysts Events	Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Rama Raju 2009	23	107	16	62	100.0%	0.83 [0.48 , 1.45]
Total (95% CI)		**107**		**62**	**100.0%**	**0.83 [0.48 , 1.45]**
Total events:	23		16			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.64 (P = 0.52)
Test for subgroup differences: Not applicable



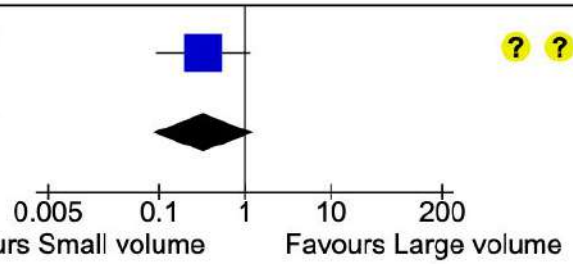
- Similar miscarriage

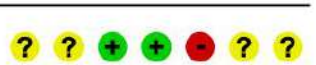
Sfontouris et al, Unpublished

Small vs large cryoprotectant loading volume Live birth

Study or Subgroup	Large volume Events	Total	Small volume Events	Total	Weight	Risk ratio M-H, Random, 95% CI
✔ Wang 2012 (1)	3	21	4	9	100.0%	0.32 [0.09 , 1.15]
Total (95% CI)		**21**		**9**	**100.0%**	**0.32 [0.09 , 1.15]**
Total events:	3		4			

Heterogeneity: Not applicable
Test for overall effect: Z = 1.74 (P = 0.08)
Test for subgroup differences: Not applicable





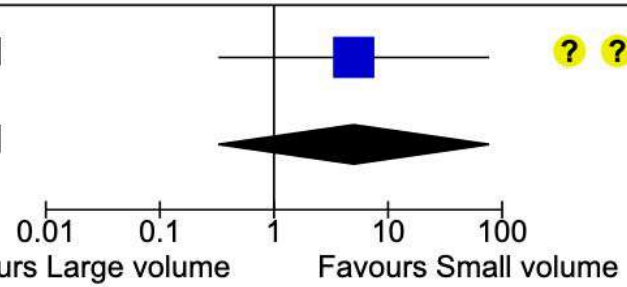
- Similar LBR – approaching significance in favour of small volume

Sfontouris et al, Unpublished

Small vs large cryoprotectant loading volume Miscarriage

Study or Subgroup	Large volume Events	Total	Small volume Events	Total	Weight	Risk ratio M-H, Random, 95% CI	Risk ratio M-H, Random, 95% CI	Risk of Bias A B C D E F G
✔ Wang 2012	3	6	0	4	100.0%	5.00 [0.33 , 76.81]		? ? + + - ? ?
Total (95% CI)		**6**		**4**	**100.0%**	**5.00 [0.33 , 76.81]**		
Total events:	3		0					

Heterogeneity: Not applicable
Test for overall effect: Z = 1.15 (P = 0.25)
Test for subgroup differences: Not applicable

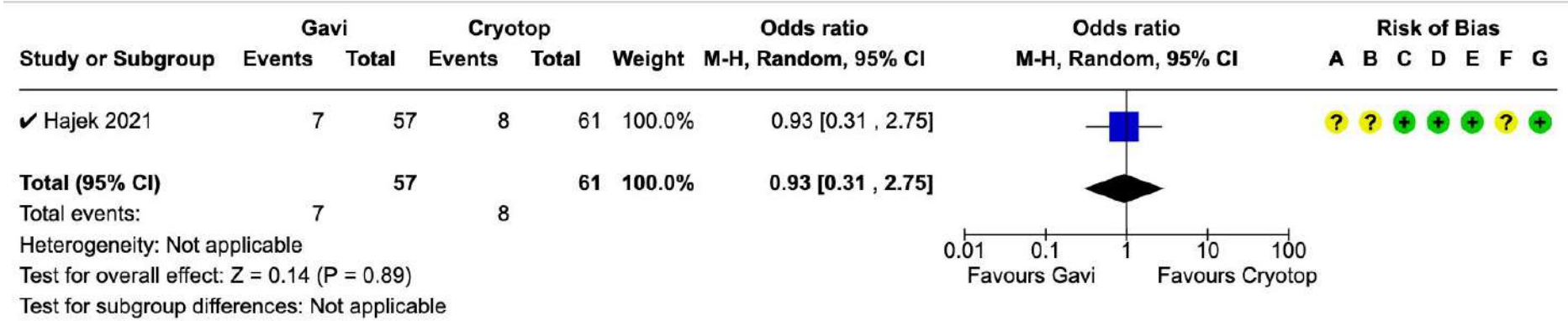


- Similar miscarriage

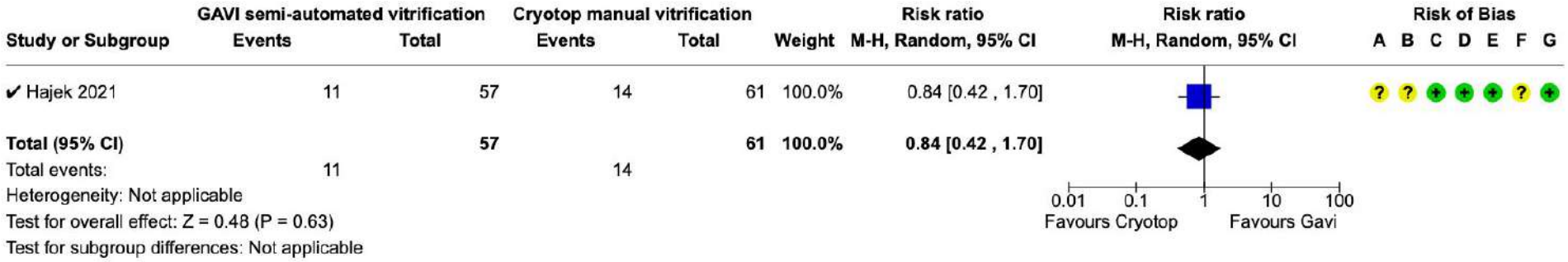
Sfontouris et al, Unpublished

Manual vs semi-automated (Gavi) vitrification

Live birth



Clinical pregnancy



- Similar LBR
- Data on miscarriage not reported

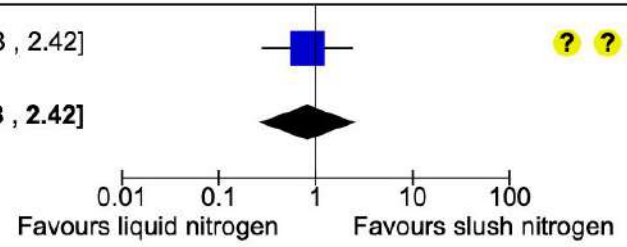
Sfontouris et al, Unpublished

Slush N_2 vs liquid N_2

Clinical pregnancy

Study or Subgroup	Slush nitrogen Events	Slush nitrogen Total	Liquid nitrogen Events	Liquid nitrogen Total	Weight	Odds ratio M-H, Random, 95% CI
✔ Klimczak 2021	20	30	22	31	100.0%	0.82 [0.28 , 2.42]
Total (95% CI)		**30**		**31**	**100.0%**	**0.82 [0.28 , 2.42]**
Total events:	20		22			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.36 (P = 0.72)
Test for subgroup differences: Not applicable

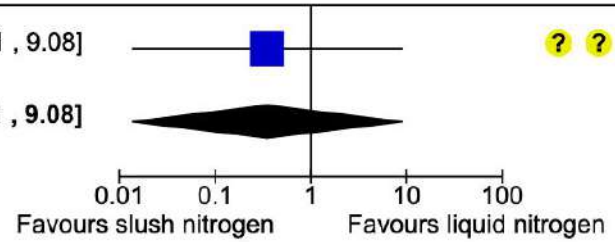


Risk of Bias: A B C D E F G

Early pregnancy loss

Study or Subgroup	Slush nitrogen Events	Slush nitrogen Total	Liquid nitrogen Events	Liquid nitrogen Total	Weight	Odds ratio M-H, Random, 95% CI
✔ Klimczak 2021	0	20	1	22	100.0%	0.35 [0.01 , 9.08]
Total (95% CI)		**20**		**22**	**100.0%**	**0.35 [0.01 , 9.08]**
Total events:	0		1			

Heterogeneity: Not applicable
Test for overall effect: Z = 0.63 (P = 0.53)
Test for subgroup differences: Not applicable



Risk of Bias: A B C D E F G

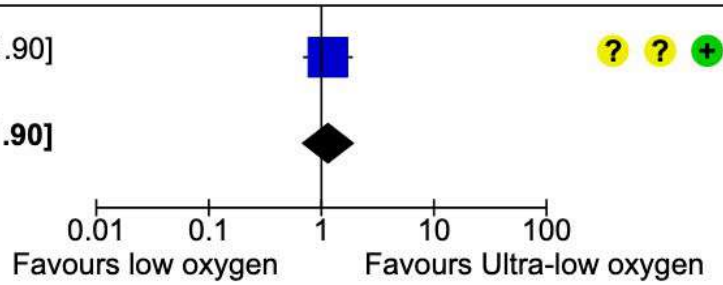
- Similar LBR
- Data on LBR not reported

Sfontouris et al, Unpublished

Low O2 (5%) vs ultra-low O2 (2%) in post-thaw culture Clinical pregnancy

Study or Subgroup	Ultra-low oxygen Events	Total	Low oxygen Events	Total	Weight	Risk ratio M-H, Random, 95% CI
✔ ElTanbouly 2019	16	30	14	30	100.0%	1.14 [0.69 , 1.90]
Total (95% CI)		**30**		**30**	**100.0%**	**1.14 [0.69 , 1.90]**
Total events:	16		14			

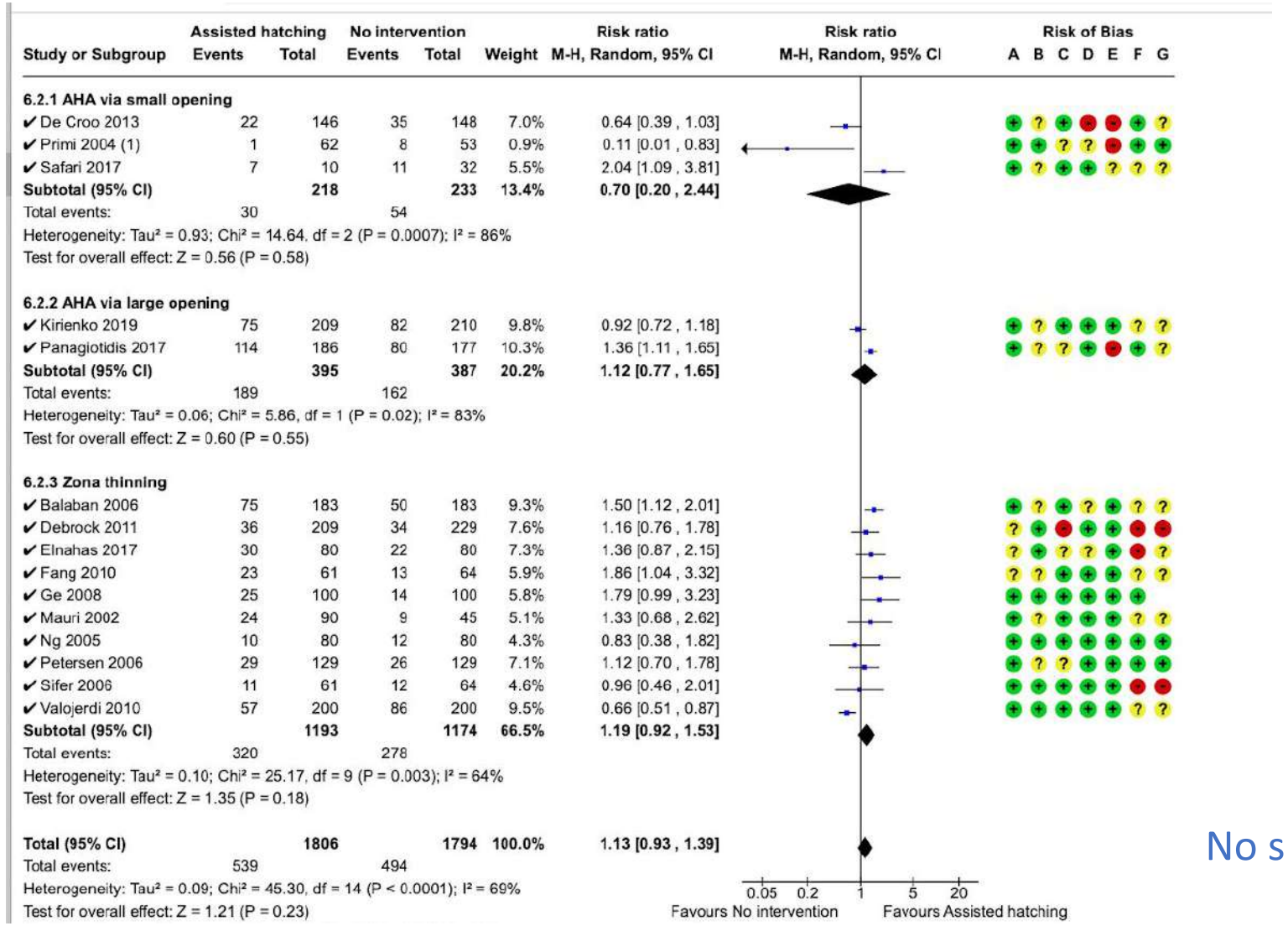
Heterogeneity: Not applicable
Test for overall effect: Z = 0.51 (P = 0.61)
Test for subgroup differences: Not applicable



- Similar CPR
- Data on LBR and miscarriage not reported

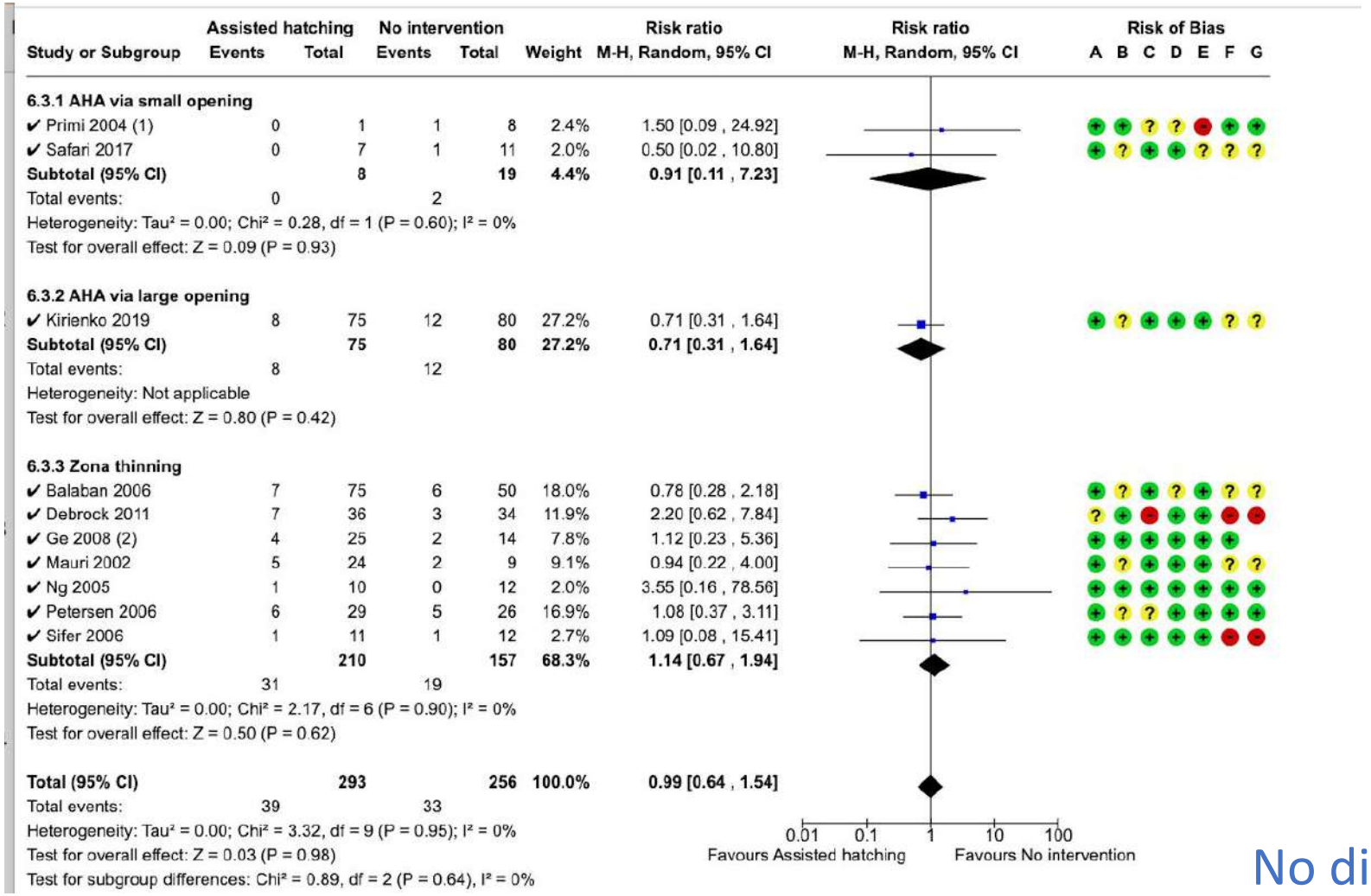
Sfontouris et al, Unpublished

Assisted hatching post-thaw – Live birth



No significant benefit of AH after warming

Assisted hatching post-thaw - Miscarriage



Study or Subgroup	Assisted hatching Events	Total	No intervention Events	Total	Weight	Risk ratio M-H, Random, 95% CI
6.3.1 AHA via small opening						
✔ Primi 2004 (1)	0	1	1	8	2.4%	1.50 [0.09 , 24.92]
✔ Safari 2017	0	7	1	11	2.0%	0.50 [0.02 , 10.80]
Subtotal (95% CI)		**8**		**19**	**4.4%**	**0.91 [0.11 , 7.23]**
Total events:	0		2			
Heterogeneity: Tau² = 0.00; Chi² = 0.28, df = 1 (P = 0.60); I² = 0%						
Test for overall effect: Z = 0.09 (P = 0.93)						
6.3.2 AHA via large opening						
✔ Kirienko 2019	8	75	12	80	27.2%	0.71 [0.31 , 1.64]
Subtotal (95% CI)		**75**		**80**	**27.2%**	**0.71 [0.31 , 1.64]**
Total events:	8		12			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.80 (P = 0.42)						
6.3.3 Zona thinning						
✔ Balaban 2006	7	75	6	50	18.0%	0.78 [0.28 , 2.18]
✔ Debrock 2011	7	36	3	34	11.9%	2.20 [0.62 , 7.84]
✔ Ge 2008 (2)	4	25	2	14	7.8%	1.12 [0.23 , 5.36]
✔ Mauri 2002	5	24	2	9	9.1%	0.94 [0.22 , 4.00]
✔ Ng 2005	1	10	0	12	2.0%	3.55 [0.16 , 78.56]
✔ Petersen 2006	6	29	5	26	16.9%	1.08 [0.37 , 3.11]
✔ Sifer 2006	1	11	1	12	2.7%	1.09 [0.08 , 15.41]
Subtotal (95% CI)		**210**		**157**	**68.3%**	**1.14 [0.67 , 1.94]**
Total events:	31		19			
Heterogeneity: Tau² = 0.00; Chi² = 2.17, df = 6 (P = 0.90); I² = 0%						
Test for overall effect: Z = 0.50 (P = 0.62)						
Total (95% CI)		**293**		**256**	**100.0%**	**0.99 [0.64 , 1.54]**
Total events:	39		33			
Heterogeneity: Tau² = 0.00; Chi² = 3.32, df = 9 (P = 0.95); I² = 0%						
Test for overall effect: Z = 0.03 (P = 0.98)						
Test for subgroup differences: Chi² = 0.89, df = 2 (P = 0.64), I² = 0%						

No difference in miscarriage rate

Quality of data

- Small number of RCTs for each technique
- High degree of heterogeneity between studies
- Low quality of data
- Still unsure whether there is a beneficial effect of any of these techniques
- Numerous observational and retrospective studies may suggest significant effects
- More well-designed RCTs are necessary

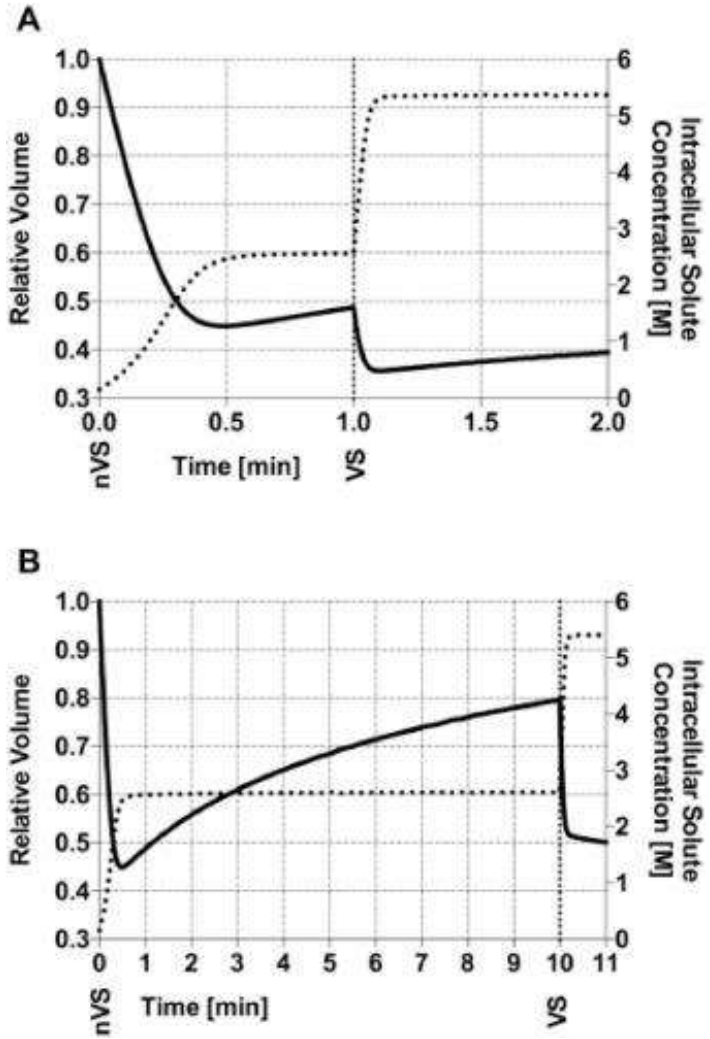
- Choice of techniques largely depends on laboratory set-up and personal preference

Summary of findings

Interventions	Outcomes
Vitrification vs slow freezing	Similar LBR and misc. RCT using blastocysts not identified
Open vs closed vitrification	Similar LBR and misc.
Artificial blastocyst shrinkage vs no intervention	Similar LBR and misc.
Laser vs mechanical blastocyst shrinkage	Laser is better
Pronuclear vs cleavage vitrification	Similar LBR and misc.
Early vs expanded blastocyst vitrification	Similar LBR and misc.
FET same day vs overnight culture	Higher LBR with overnight culture. Similar misc.
Small vs large loading volume	Trend for higher LBR (NS) with small volume. Similar misc.
Manual vs semi-automated vitrification	Similar CPR and LBR
Slush N_2 vs liquid N_2	Similar CPR and EPL
Low O2 (5%) vs ultra-low O2 (2%)	Similar CPR
Assisted hatching vs no intervention	Similar LBR and misc.

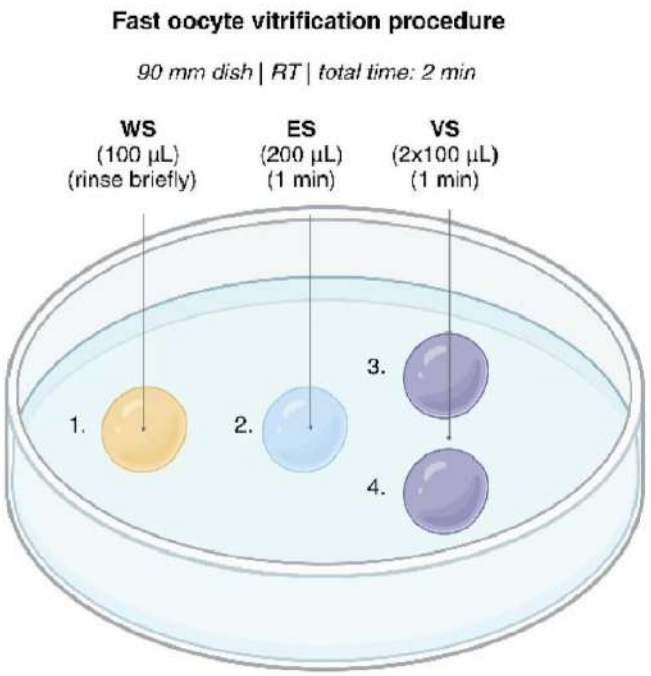
Ultra-fast freezing and warming

- Gallardo et al. (2019): simulated oocyte osmotic behaviour to cryoprotectant solution
- Equilibrium can be achieved in much shorter times (3—60 sec) similarly to traditional exposure (9-15 min).
- Showing feasibility of ultra-fast vitrification

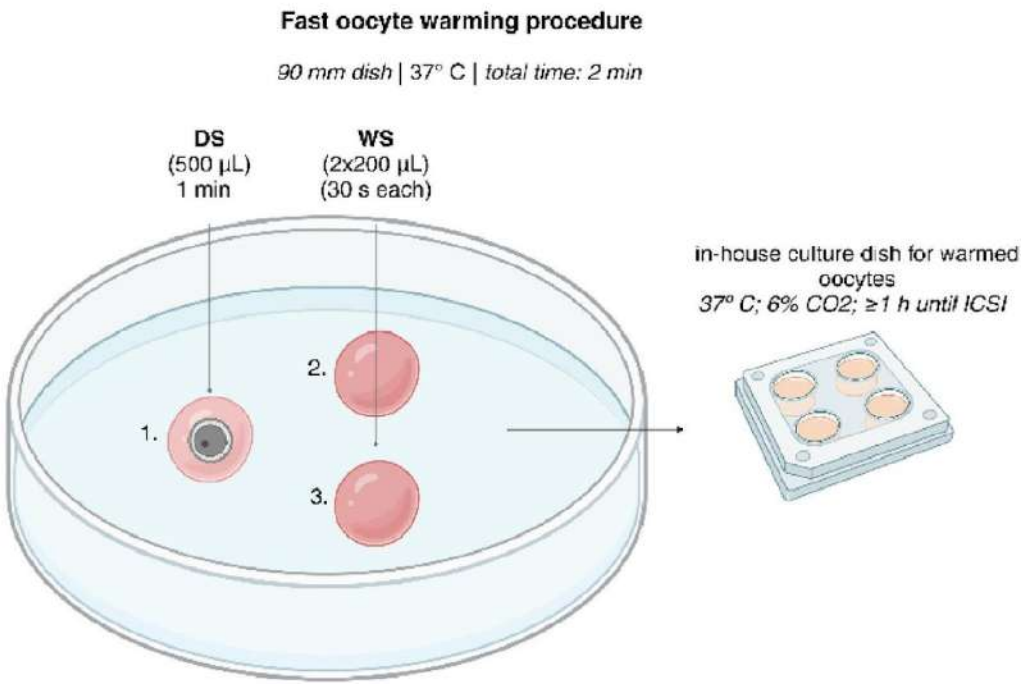


Oocyte ultra-fast freeze and warming

A.

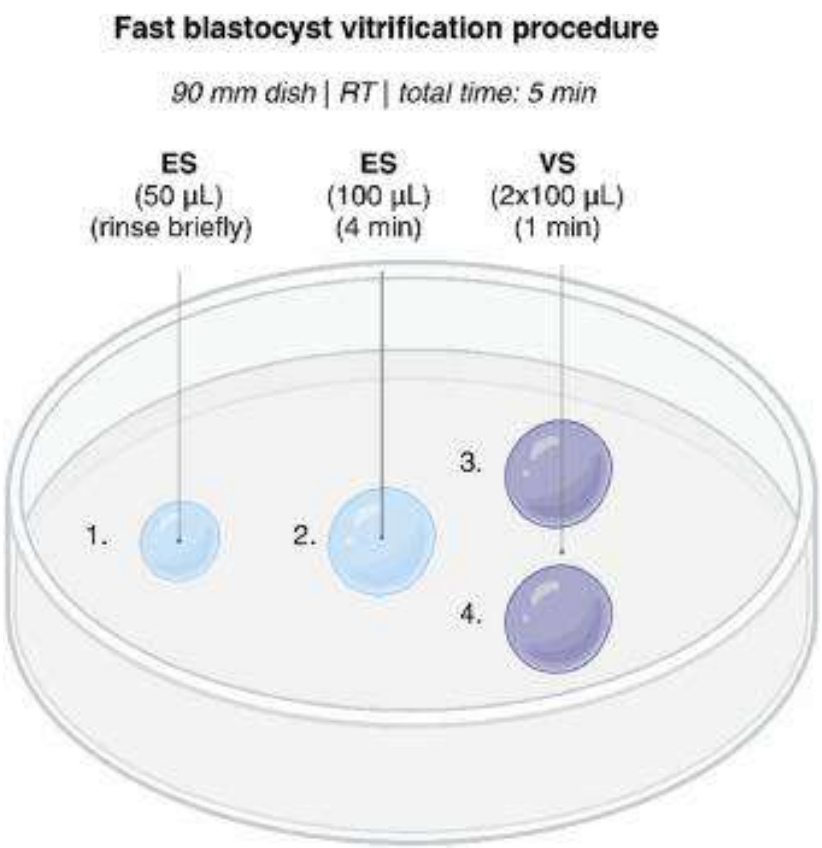


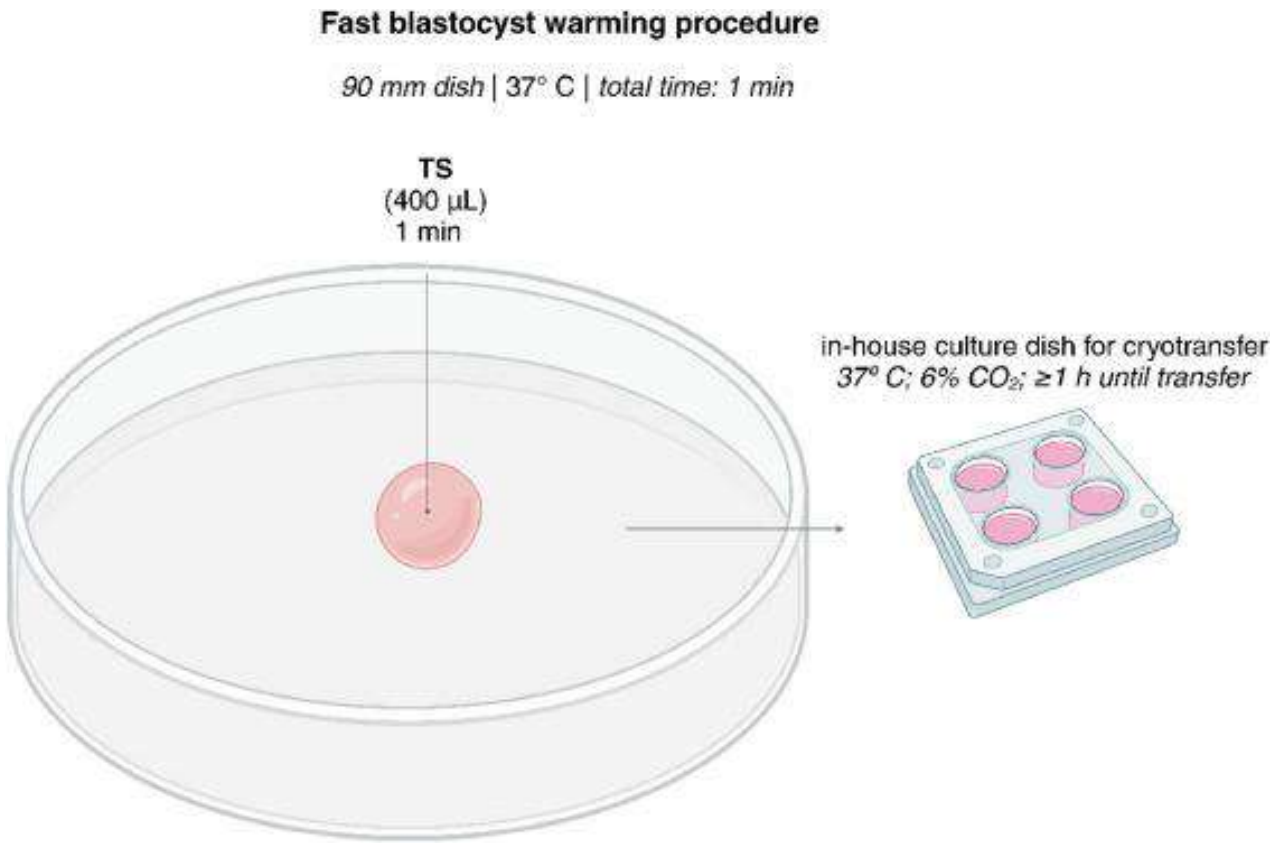
B.



Martinez-Rodero et al 2025 RBMO

Blastocyst ultra-fast freeze and warming

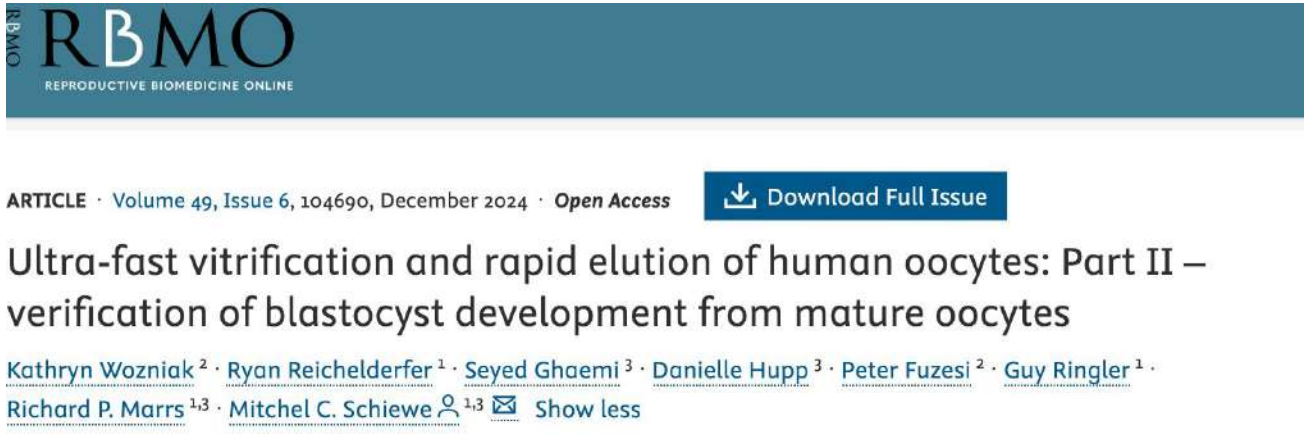




Fast and furious blastocyst rehydration

- Similar survival rate, similar CPR
- Higher ongoing pregnancy using fast warming vs multi-step warming
- Lower miscarriage rate using fast warming
- Shorter time using fast warming

Ultra-fast vitrification and warming of oocytes



- Studies using in-vitro matured MI/GV oocytes
- >95% survival with ultra-fast protocol (Liebermann 2024)
- Higher survival with ultra-fast (98%) compared to standard protocol (83.3%) (Wozniak 2025)
- Benefit of reduced exposure to room temperature, and shorter time of procedure.

Universal embryo warming

RBMO
REPRODUCTIVE BIOMEDICINE ONLINE

ARTICLE · Articles in Press, 104923, March 09, 2025 · *Open Access*

Universal post-warming dilution of vitrified embryos: impact of different vitrification/warming kits, warming volume and rapid dilution/ rehydration steps on survival and clinical outcomes

Lodovico Parmegiani [1] · Gabor Vajta [2,3] · Colleen Lynch [4] · Alessandra Arnone [1] · Silvia Bernardi [1] · Antonio Manuel Maccarini [1] · Sara Lanzilotti [1] · Azzurra Rastellini [1] · Enzo Troilo [1] · Elena Nardi [5] · Walter Ciampaglia [1]

Table 1 LCS2 – Combinations of different kits. Number of warming cycles per group

Vitrification kit used	Group IFU -S	Group USSW- I	Group USSW - K	Group USSW - S	Group USSW - V
Kitazato - *vitrification media VT601*	11	48	2	20	3
Sage - *vitrification kit ART-8026*	6	31	3	11	5
Fuji Film Irvine - *Vit Kit Freeze 90133-SO*	5	16	0	7	2
Total	**22**	**95**	**5**	**38**	**10**

- Possible to combine various kits for vitrification/warming
- Possible to perform the universal single-step warming (USSW) with any warming kit brand.

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Table 2 LCS1 – Outcome measures (Survival rate, Clinical Pregnancy rate, Embryo Implantation rate, Live Birth rate)

	Group KK	Group KS	Group KI	Group SK	Group SS	Group SI	Group IK	Group IS	Group II	Group HK	Group HS	Group HI
Mean female age (± SD) at warming	37.6±4.5	37.2±4.3	37.9±4.3	38.0±5.2	37.5±5.1	37.6±5.0	38.1±4.7	37.5±5.3	37.0±4.7	37.1±4.0	37.3±5.2	37.1±4.2
Survival % (No. of surviving embryos/ warmed embryos)	100% (237/237)	99.7% (307/308)	99.7% (285/286)	99.5% (189/190)	99.8% (531/532)	99.8% (531/532)	100% (70/70)	100% (229/229)	100% (292/292)	100% (134/134)	100% (110/110)	100% (124/124)
Clinical Pregnancy % (Presence of a gestational sac / No. embryo transfers)	39% (78/200)	39.6% (103/260)	40.8% (95/233)	47.0% (70/149)	41.4% (173/418)	36.1% (108/299)	36.2% (21/58)	36.4% (67/184)	38.2% (89/233)	37.8% (34/90)	34.5% (29/84)	36% (32/89)
Embryo Implantation % (No. of gestational sacs/ transferred embryos)	35% (83/237)	37.5% (115/307)	35.1% (100/285)	39.7% (75/189)	35.4% (188/531)	33.1% (124/375)	31.4% (22/70)	31.4% (71/226)	33.8% (99/292)	29.9% (40/134)	29.1% (32/110)	29% (36/124)
Live Birth % (No. of pregnancies leading to births / embryo transfers)	32.5% (65/200)	31.2% (81/260)	27.0% (63/233)	34.9% (52/149)	30.1% (77/299)	27.8% (83/299)	27.6% (16/58)	29.3% (54/184)	28.8% (67/233)	28.9% (26/90)	27.4% (23/84)	29.2% (26/89)
No. of babies born	70	87	64	56	139	97	17	59	74	29	24	29

P-Value NS. Exceptions (mean female age at Mann-Whitney Rank Sum Test): KI (37.9 ± 4.3) vs SS (37.5± 5.1) P=0.040, vs II (37.0± 4.7) P=0.012

Age

Survival

Clinical pregnancy

Implantation

Live birth

Babies

New methods of vitrification
Ultra-fast and universal warming

- Higher efficiency
- Flexibility
- Time-saving
- Improved workflow in the lab
- Proof of concept successful
- Time to move to prospective clinical studies

Thank you!