Oocyte and Embryo Cryopreservation Technical and Legal Approaches

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OVERVIEW

1. **Technical &Legal Approaches for Oocyte Cryopreservation**
   - **Technical:** Cryopreservation techniques, indications, clinical and neonatal outcome
   - **Legal:** Current situation in Turkey
2. **Technical &Legal Approaches for Embryo Cryopreservation**

Technical: Cryopreservation techniques, indications, clinical and neonatal outcome

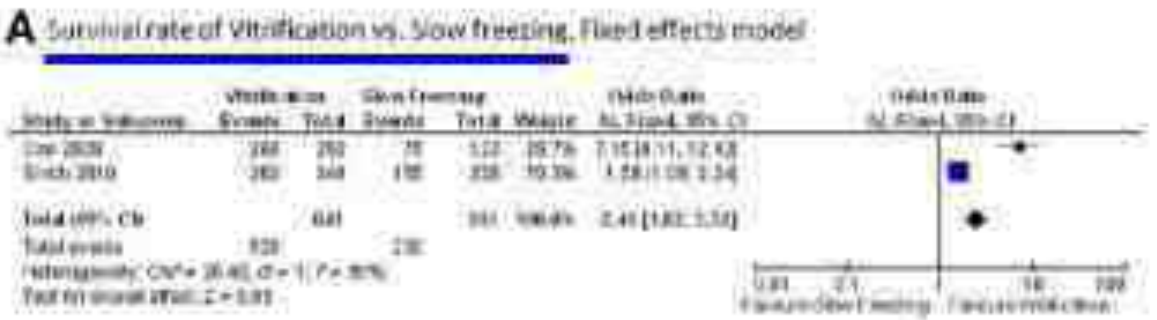
Legal: Current situation in Turkey

1. Technical Approaches for Oocyte Cryopreservation

Clinical Application of Oocyte Vitrification: Review & Meta-analysis Slow freezing or Vitrification????

FIGURE 2

Odds ratio of postthawing/warming oocyte survival rate after vitrification and SF. (A) Fixed-effects model. (B) Random-effects model.

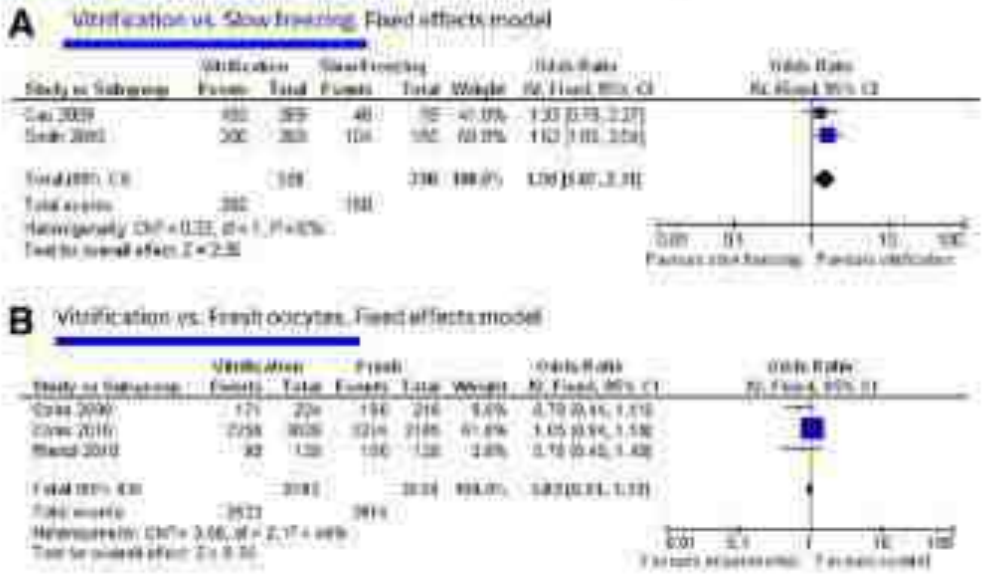


Cobo &Diaz F&S 2011

Clinical Application of Oocyte Vitrification: Review & Meta-analysis Slow freezing or Vitrification????

FIGURE 3

Odds ratio of fertilization rate. (A) Vitrification versus SF. (B) Vitrification versus fresh oocytes. Fixed effects-model.



Cobo. Meta-analysis of the use of oocyte vitrification. Fertil Steril 2011

Cobo &Diaz F&S 2011

MII Oocyte Cryopreservation : ASRM Guideline

TABLE 1

Summary of randomized controlled trials comparing fresh versus vitrified oocytes.

	Cobo 2008 (24)	Cobo 2010 (26)	Rienzi 2010 (25)	Parmegiani 2011 (19)
Patient population	Oocyte donors	Oocyte donors	Infertile patients <38 years of age requiring ICSI with ≥6 mature oocytes	Infertile patients <40 years of age requiring ICSI with ≥5 mature oocytes
No. patients	30 vitrification 30 fresh	235 vitrification 199 fresh	42 vitrification 40 fresh	31 vitrification 31 fresh
Mean age at retrieval	26	28	35	35
No. oocytes	231 vitrification 219 fresh	3266 vitrification 3185 fresh	124 vitrification 120 fresh	158 vitrification NA fresh
No. oocytes per retrieval	18.2	11	13	NA
Survival	96.9%	92.5%	96.8%	89.9%
Fertilization rate	76.1 vitrification 83.7 fresh	74% vitrification 73% fresh	79.3% vitrification 82.3% fresh	71% vitrification 72.6% fresh
No. transferred vitrification vs. fresh	3.8 vitrification 3.9 fresh	1.7 vitrification 1.7 fresh	2.3 vitrification 2.5 fresh	2.5 vitrification 2.6 fresh
Day of transfer	3	3	3	2–3
Implantation rate	47.8% vitrification 100% fresh	39.9% vitrification 40.9% fresh	26.4% vitrification 21.7% fresh	17.1% vitrification NA fresh
CPR/transfer vitrification vs. fresh	60.8% (23 vitrification transfers) 100% (11 fresh transfer)	55.4% vitrification 53.6% fresh	38.5% vitrification 42.5% fresh	35.5% vitrification 16.2% fresh
OHSS/oocyte thawed	0.1%	4.5%	12%	4.5%

Note: All used vitrification with Cryotop, 72% ES + 11% DMSO + 0.5M sucrose. CPR = clinical pregnancy rate.

Practice Committee. Oocyte cryopreservation. Web Ref# 2012.

***Trend toward better results with vitrification, only 1 RCT by Smith.et al. 2010 compares vit.&slow shows sig. higher CPR with vit.

ASRM F&S 2013

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

Alpha Scientists in Reproductive Medicine [1,*]

Reproductive BioMedicine Online (2012) 25, 146–167

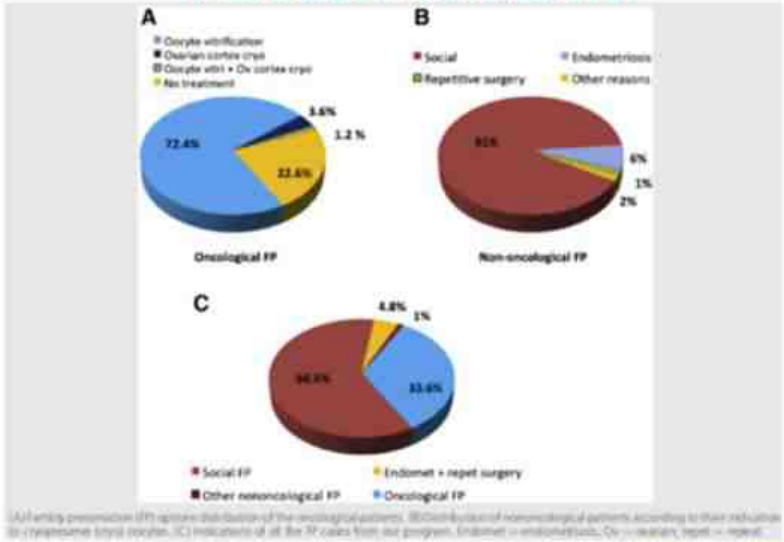
Table 1 Oocyte key performance indicator values.

KPI		Competence		Benchmark
O1	Morphological survival	Freezing	>50%	75%
		Vitrification	70%	85% (95% for donors <30 years)
O2	Fertilization rate	No more than 10% (absolute; i.e. 10 percentage points) lower than that for the comparable population of fresh oocytes at the centre		
O3	Embryo development rate	Freezing	No more than 10–30% (relative) lower than that for the comparable population of fresh embryos at the centre	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	
O4	Implantation rate	No more than 10–30% (relative) lower than that for the comparable population of fresh embryos at the centre		

Oocyte Vitrification

- Fertility preservation- Medical Reasons
- Cancer patients
- Other medical conditions compromising fertility;
- * Endometriozis
- * High risk for early ovarian failure
- Fertility preservation -social (non-medical) reasons
- * Elective freezing age related decline of fertility to postpone motherhood

Five years' experience using oocyte vitrification to preserve fertility for medical and nonmedical indications



Velasco F&S 2013

Clinical outcome of nononcological FP patients.

	Nononcological	Oncological
No. of patients/warming cycles	26	4
"Fresh" ETs (%)	24 (92.3)	4
No. of embryos transferred	37 (1.5 ± 0.6)	8 (2)
CPR/patient (%)	11 (42.3)	1 (25)
OPR/patient (%)	8 (30.7)	1 (25)
No. of patients with surplus embryos	17 (65.3)	2 (50)
No. of surplus embryos vitrified	49 (2.8 ± 4.2)	4 (2)
No. of cryotransfers	15 (88.2)	1
No. of embryos transferred per cryotransfer	2.3 ± 0.7	2
CPR/patient (%)	7 (46.6)	1 (100)
OPR/patient (%)	5 (33.3)	0
Total live birth	5	1
Mean birth weight (g)	3,150 ± 0.3	3,440
Sex of the baby		
Female (%)	3 (60)	0
Male (%)	2 (40)	1

Note: Unless otherwise indicated, values are mean ± SD. CPR = clinical pregnancy rate; FP = fertility preservation; OPR = ongoing pregnancy rate.

Velasco F&S 2013

Fertility Preservation for Cancer Patients

Live births reported for cancer patients; Slow freezing & Vitrification

Clinical outcomes and live births reported in cancer patients who pursued fertility through oocyte cryopreservation (slow freezing and vitrification).

	Yang et al., 2007 (158)	Porcu et al., 2008 (159)	Sánchez Serrano et al., 2009 (160)	Kim et al., 2011 (161)	García-Velasco, 2013 (12)
Type of malignancy	Hodgkin lymphoma	Borderline ovarian tumor	Breast cancer	Chronic myeloid leukemia	Non-Hodgkin lymphoma
Cryopreservation technique	Slow freezing	Slow freezing	Combined OTC/SF + OV (Cryotop)	Vitrification (EMS)	Vitrification (Cryotop)
Age at FP, y	27	26	36	32	31
No. of cryopreserved oocytes	13	7	16	7	4
Storage time, y	4	4	2	4	2
Twin or single pregnancy	Single*	Twin	Twin	Single	Single
No. of live births	1	2	1	1	1
Weeks of gestation	37	38	34	39 + 1 d	39
Weight of baby, g	3,062	2,100 and 2,400	1,690 and 1,810	2,810	3,440
Sex of baby	Male	Females	Males	Male	Male

* Gestational carrier.

Slow freezing no.of live birth 3
Vitrification 4

Cobo et al., F&S 2013

Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial

Ana Cobo*, Marcos Meseguer, José Remohí, and Antonio Pellicer

Table III Clinical outcome according to the type of oocytes received

	Egg-bank	Fresh
Number of embryos transferred	267 (90.5)	259 (89.6)
Mean number of embryos replaced	3.13 (1.34 ± 0.7)	4.98 (1.72 ± 0.7)
Number of cycles with embryo re-vitrification/cryopreservation	196 (68.7)	216 (74.7)*
Mean number of re-vitrified or cryopreserved embryos	5.92 (2.0 ± 2.1)	7.45 (2.5 ± 2.3)*
Implantation rate	205 (39.9)	264 (40.9)
Positive hCG test/cycle	165 (55.9)	159 (55.0)
Clinical pregnancy rate/cycle	148 (50.3)	144 (49.8)
Positive hCG test/transfer	165 (61.8)	159 (61.4)
Clinical pregnancy rate/transfer	148 (55.4)	144 (55.6)
Twin pregnancy rate	48 (32.4)	54 (37.5)

Unless otherwise indicated values are mean ± SD or n (%).
*P < 0.05.

Table IV Primary outcome, OPR, according to the type of oocytes received.

	Egg-bank	Fresh
Ongoing pregnancy rate/ITT	131 (43.7)	125 (41.7)
Ongoing pregnancy rate/cycle	131 (44.4)	125 (43.3)
Ongoing pregnancy rate/transfer	131 (49.1)	125 (48.3)

Unless otherwise indicated values are mean ± SD or n (%).
ITT, intention to treat.

Human Reproduction, Vol.25, No.9 pp. 2239–2246, 2010

Fresh Cycles	Fresh oocytes (n = 99)	Vitrified oocytes (n = 99)
Transferred embryos (mean ± SD)	1.82 ± 0.44	1.90 ± 0.34
Clinical pregnancy rate/transfer (%)	47 (47.5)	53 (53.5)
Implantation rate (%)	33.3	34.0
Ongoing pregnancy rate/transfer (%)	39 (39.4)	44 (44.4)
Miscarriage rate (%)	9 (19.1)	11 (20.8)
Live birth rate/transfer (%)	38 (38.4)	42 (43.4)
Multiple pregnancy rate (%)	27.7	20.8

Cryo cycles	Fresh oocytes	Vitrified oocytes
Number of cryotransfer cycles	55	30
Total thawed embryos	187	85
Thawed embryos/recipient (mean ± SD)	3.46 ± 1.73	2.83 ± 1.64
Survival rate (%)	70.1	65.8
Cycles without embryo transfer	6	6
Cycles with embryo transfer	49	24
Transferred embryos (mean ± SD)	1.98 ± 0.63	1.64 ± 0.81
Clinical pregnancy/transfer (%)	40.8	33.3
Implantation rate (%)	21.8	26.8

NO statistical differences in terms of clinical outcome both for fresh and cryopreservation cycles

Sole et al., HR 2013

Delivery rates after oocyte vitrification: Multicentric study

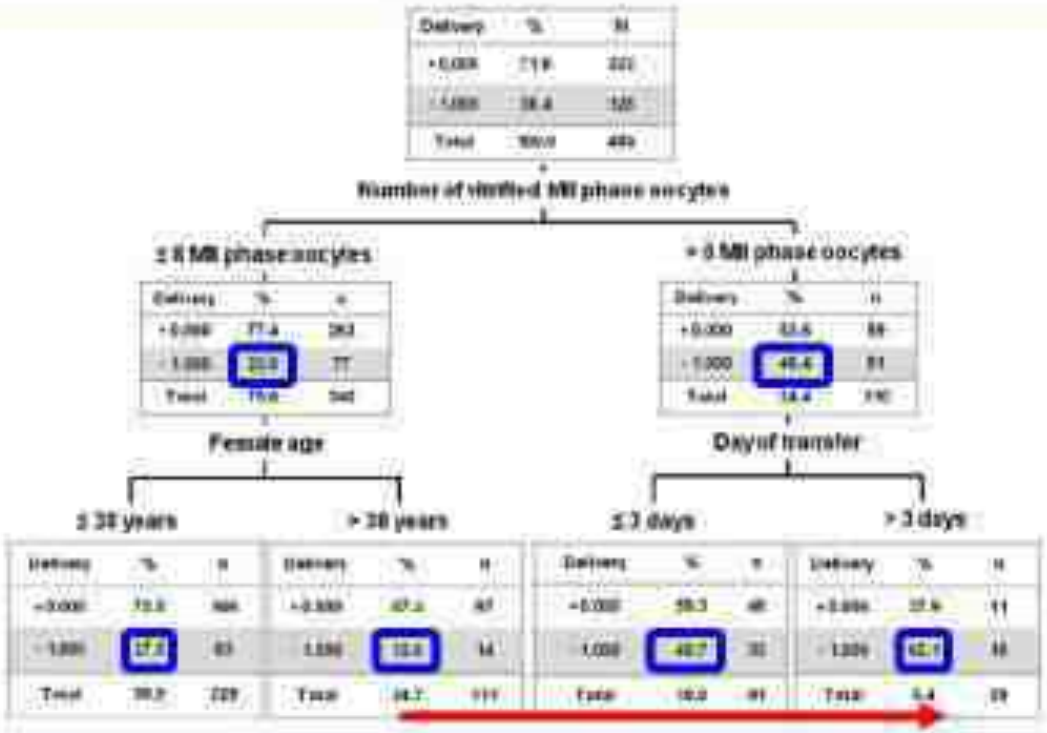


Figure 1

Observational longitudinal cohort multicentric study.
486 cycles, 2721 oocytes warmed,3 ART centers

Rienzi et al., HR 2012

Oocyte vitrification for low-responders? Accumulation of oocytes

Table 3 Live birth rate per patient and per embryo transfer.

	LR-Accu-Vit	LR-fresh
Embryo transfers (n)	220	318
Transfer cancellations/patient (%, 95% CI)	9.1 (6.8–11.4) [a]	34.0 (29.8–38.2) [b]
Implantation rate		
n/total	110/440	138/540
% (95% CI)	25.0 (20.7–30.0)	25.6 (21.9–29.3)
Embryos transferred (mean, 95% CI)	2.0 (1.9–2.1) [a]	1.7 (1.6–1.8) [b]
Live-birth rate/embryo transfer		
n/total	71/220	108/318
% (95% CI)	33.2 (25.7–38.0)	34.0 (28.7–39.1)
Live-birth rate/patient		
n/total	72/242	108/482
% (95% CI)	30.2 (24.3–35.9)	22.4 (18.7–26.1)
Cumulative live-birth rate/patient*		
n/total	88/242	114/482
% (95% CI)	36.4 (30.3–42.4) [a]	23.7 (19.9–27.4) [b]

LR Accu-Vit = low response, accumulation of oocytes and vitrification; LR-fresh = low response, fresh oocytes.

[a,b] Same superscript letters in a row indicate a statistically significant difference ($P < 0.05$).

*Calculated considering the additional number of babies born after subsequent embryo cryotransfers.

No.POR:242, <5 oocytes.

Decision of no.of accum cycles(2 or more):

**Total no of oocytes likely to be available after vit. (estimated survival rate)

**Need for a total no.of 5 emb.for ET inconsecutive cycles (no.needed to reach 52 % CLBR;standard for normoresponders.

**patient's own decision

Cobo et al., RBM Online 2012

Safety of Oocyte Vitrification???

Characteristic	All pregnancies (n = 453)	Singleton pregnancies (n = 217)	Multiple gestation pregnancies (n = 36)
Mean gestational age (weeks + days)	37 + 3	37 + 3	35 + 4
No. of deliveries at 34–37 weeks (%)	60 (30)	30 (22)	16 (57)
No. of deliveries at <34 weeks (%)	10 (5)	9 (4)	4 (14)
	All newborns (n = 280)	Singleton newborns (n = 242)	Multiple gestation newborns (n = 38)
Birth weight (mean ± SD) (g)	2784 ± 57	2839 ± 57	2258 ± 55
No. of LBW (%)	68 (24)	34 (17)	34 (78)
No. of VLBW (%)	4 (2)	1 (0.5)	3 (7)
Median Apgar score at 1 min	8	8	8
Median Apgar score at 5 min	10	10	10
Incidence of congenital anomalies			
Biliary atresia	1	0	1
Cleft foot	1	1	0
Skin hemangioma	1	1	0
Ventricular septal defect	2	0	2
Total (%)	5 (1.8)	2 (0.8)	3 (7.9)

LBW = low birth weight; VLBW = very low birth weight.

Chian et al., RBM Online 2008.

Group characteristics	OS: Singleton pregnancies (n = 9)	OS: Multiple gestation pregnancies (n = 9)
Mean gestational age (weeks + days)	39 + 1	36 + 4
No. of delivery 34–37 weeks (%)	0	4 (66.7)
No. of delivery <34 weeks (%)	0	0
	Singleton newborns (n = 9)	Multiple gestation newborns (n = 13)
Mean birth weight (g ± SD)	3183.7 ± 376.9	2277.9 ± 395.7
No. of LBW (1500 to 2500 g) (%)	0	9 (69)
No. of VLBW (<1500 g) (%)	0	0
Males	7	4
Females	2	9

Note: LBW: low birth weight; VLBW: very low birth weight.

No congenital abn. for 22 babies — Chian et al., F&S 2008

4/489 infants born had birth defects, Cobo et al.RBM 2011

- 2/147 infants born had congenital abn. Rienzi et al.,HR 2012

---Effect of oocyte vit in the metabolomic profile of embryos developed had shown no stat. sig. diff. when compared with fresh group. Oocyte vit. does not disturb embryonic metabolic profiles. Dominguez et al., F&S 2013

There's no increased of aneuploidy for blastocysts from vitrified oocytes by microarray-based CCS on trophoectoderm biopsy, Forman et al., F&S 2012

30 Eylül 2014 Sayı : 29135

Sağlık Bakanlığı

ÜREMEYE YARDIMCI TEDAVİ UYGULAMALARI VE ÜREMEYE YARDIMCI TEDAVİ MERKEZLERİ HAKKINDA YÖNETMELİK

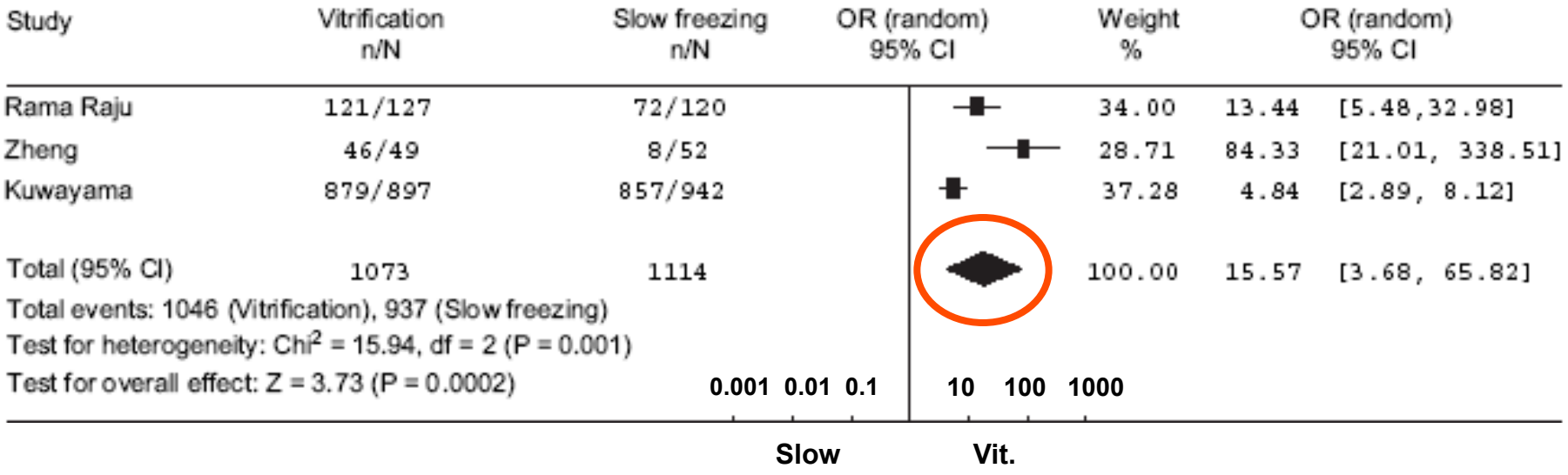
- **BEŞİNCİ BÖLÜM**

- **Hizmet Sunumuna İlişkin Diğer Esaslar**
- **Üreme hücreleri ve gonad dokularının saklanma kriterleri**
- **MADDE 20**
- **(3) Kadınlarda üreme hücreleri ve gonad dokularının saklanmasını gerektiren tıbbî zorunluluk halleri şunlardır;**
- **a) Kemoterapi ve radyoterapi gibi gonad hücrelerine zarar veren tedaviler öncesinde,**
- **b) Üreme fonksiyonlarının kaybedilmesine yol açacak olan ameliyatlar (yumurtalıkların alınması gibi operasyonlar) öncesinde,**
- **c) Düşük over rezervi olup henüz doğurmamış veya aile öyküsünde erken menopoz hikâyesinin üç uzman tabipten oluşan sağlık kurulu raporu ile belgelendirilmesi durumunda.**

Cryopreservation of human embryos by vitrification or slow freezing: A systematic review and meta-analysis

FIGURE 2

Odds ratio of postthawing survival rate of cleavage stage embryos after vitrification and slow freezing.



Pubmed search: 873, only 3 included!!,
Primary outcome: Postthaw survival rate,
Sec.Outcome: Cleavage&Blastocyst dev.& hatching, CPR

Pooled data on cleavage, blastocyst development &hatching, CPR, IR, and LBR were NOT feasible

Loutradi et al., F&S 2008

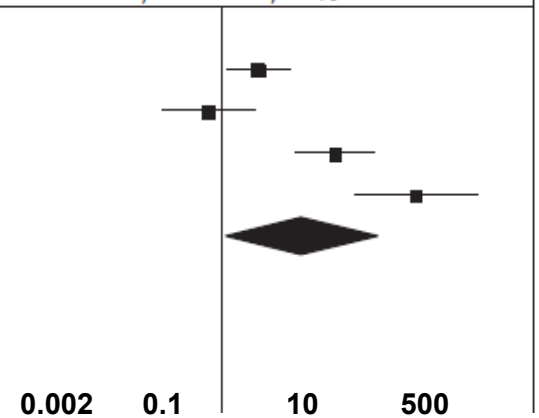
Cryopreservation of cleavage stage embryos by vitrification vs. slow freezing?? Which one is better?

Survival

Study or Subgroup	Vitrification Events	Vitrification Total	Slow freezing Events	Slow freezing Total	Weight	Odds Ratio M-H, Random, 95% CI
2.1.1 Cleavage stage embryos						
Balaban et al., 2008	222	234	206	232	17.8%	2.33 [1.15, 4.75]
Li et al., 2007	71	80	73	80	16.0%	0.76 [0.27, 2.14]
Rama Raju et al., 2005	121	127	72	120	16.8%	13.44 [5.48, 32.98]
Zheng et al., 2005	46	49	8	52	14.0%	84.33 [21.01, 338.51]
Subtotal (95% CI)		490		484	64.6%	6.35 [1.14, 35.26]
Total events	460		359			

Heterogeneity: $Tau^2 = 2.78$; $Chi^2 = 37.80$, df = 3 ($P < 0.00001$); $I^2 = 92\%$

Test for overall effect: Z = 2.11 (P = 0.03)



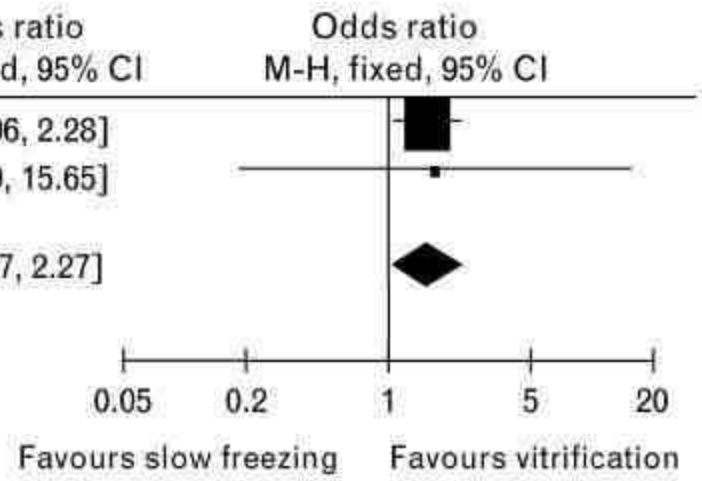
Blastocyst development

Study or subgroup	Vitrification Events	Vitrification Total events	Slow freezing Total weight	Odds ratio M-H, fixed, 95% CI
Balaban *et al.*, 2008	134	222	102 206 96.8%	1.55 [1.06, 2.28]
Zheng *et al.*, 2005	9	46	1 8 3.2%	1.70 [0.19, 15.65]
Total (95% CI)		268	214 100.0%	1.56 [1.07, 2.27]
Total events	143		103	

**Biopsied embs.

Heterogeneity: $Chi^2 = 0.01$, df = 1 ($P = 0.94$); $I^2 = 0\%$

Test for overall effect: $Z = 2.30$ ($P = 0.02$)



Li and Rama Raju found no stat sig. dif for CPR

Efstratios et al.,Cur.Op.Obs&Gynec. 2009

Table 1 Characteristics of included studies.

Study	Country	Study period	Interventions	Method of randomization	No. of participants (No. of cycles)	Financial support
Bernal et al. (2008)	USA	January 2007–April 2008	Vitrification versus slow programmed freezing	Alternate randomization	115 women (64:51)	No external financial support
Kim et al. (2000)	Korea	January 1998–July 1999	Vitrification versus slow programmed freezing	Unclear	Unclear (42:216)	None declared
Li et al. (2007)	China	June 2005–March 2007	Vitrification versus slow programmed freezing	Alternate randomization	80 women (40:40)	None declared
Mauri et al. (2001)	Brazil	January 1991–March 2000	Ultra-rapid freezing versus slow programmed freezing	Computer-generated randomization list	160 women (80:80)	No external financial support
Rama Raju et al. (2005)	India	May 2004–June 2005	Vitrification versus slow programmed freezing	Unclear	164 women (84:80)	None declared
Van den Abbeel et al. (1997a)	Belgium	October 1992–March 1993	Ultra-rapid freezing versus slow programmed freezing	Randomization list	Unclear (100:100)	Belgium National Fund for Medical Research

Current meta-analysis indicate that embryo vitrification is superior to slow freezing based on direct comparison of embryo survival and CPR,OPR, IR were also higher

**4 Vit& SF, 2 UF& SF

Desai et al.,RBM Online 2010

Outcome of vitrified cleavage-stage embryos: 1872 cycles

Overall outcome according to developmental stage at vitrification.

	D2		D3	
	n (%)	95% CI (%)	n (%)	95% CI (%)
No. of warming cycles	147		1,725	
No. of warmed embryos	497		3,491	
Age (y)	36.1 ± 4.0[a]	(35.6–36.4)	37.4 ± 5.4[a]	(36.7–37.8)
Survival rate	472 (94.9)[a]	(93.0–96.8)	3,289 (94.2)[a]	(93.4–94.9)
No. of embryo transfers (%)	135 (91.8)[a]	(87.4–96.2)	1,685 (97.6)[b]	(96.9–98.3)
No. of embryos replaced (mean ± SD)	280 (1.9 ± 0.8)[a]	(1.9–2.0)	3,057 (1.8 ± 0.6)[a]	(1.8–1.9)
Implantation rate	76 (27.2)[a]	(22.0–32.4)	1,058 (34.6)[b]	(32.9–36.3)
CPR/cycle	59 (40.1)	(32.2–48.0)	708 (41.0)	(38.7–43.3)
CPR/transfer	59 (43.7)	(35.3–52.1)	708 (42.0)	(39.6–44.4)
OPR/cycle	44 (29.9)	(22.5–37.3)	571 (33.1)	(30.1–35.3)
OPR/transfer	44 (32.6)	(24.7–40.6)	571 (33.9)	(31.6–36.1)
Miscarriage rate	15 (23.1)	(12.4–33.9)	123 (17.3)	(14.5–20.1)
DR/cycle	44 (29.9)	(22.5–37.3)	570 (33.0)	(30.9–35.3)
LBR/cycle	52 (35.3)[a]	(27.6–43.0)	677 (39.2)[a]	(36.9–41.5)

Note: CPR = clinical pregnancy rate; OPR = ongoing pregnancy rate; DR = delivery rate; LBR = live birth rate.
[a,b,c] Different superscripts in the same row indicate statistical difference ($P < .05$).

Cobo et al., F&S 2012

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

Alpha Scientists in Reproductive Medicine [1,*]

Reproductive BioMedicine Online (2012) 25, 146–167

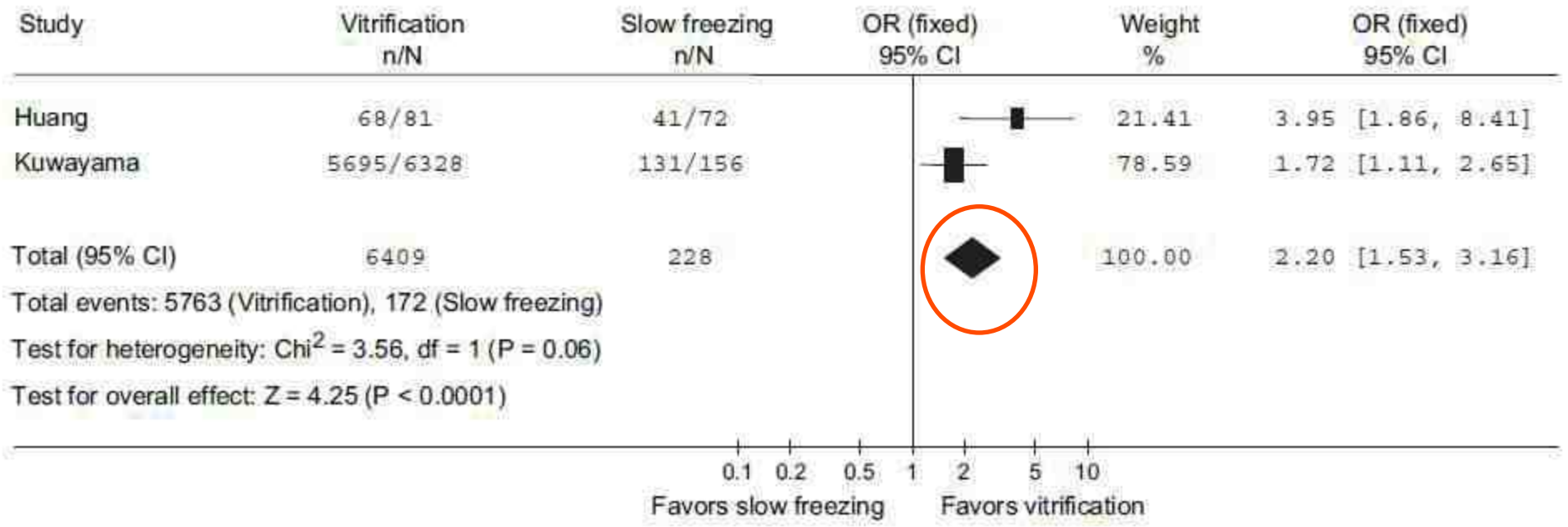
Table 3 Embryo key performance indicator values.

KPI			*Competence*	*Benchmark*
E1	Morphological survival: fully intact	Freezing	40%	55%
		Vitrification	70%	85%
E2	Morphological survival: ≥50% intact	Freezing	60%	85%
		Vitrification	85%	95%
E3	Post-thaw development (including implantation rate) for fully intact embryos		≤10% (relative) lower than that for the comparable population of fresh embryos at the centre	The same as for the comparable population of fresh embryos at the centre

The KPI values are calculated as the proportion of all thawed/warmed embryos.

Cryopreservation of blastocysts by vitrification or slow freezing: A systematic review and meta-analysis

Odds ratio of postthawing survival rate of blastocysts after vitrification and slow freezing.



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Pooled data on cleavage, blastocyst development &hatching, CPR, IR, and LBR were NOT feasible

Loutradi et al., F&S 2008

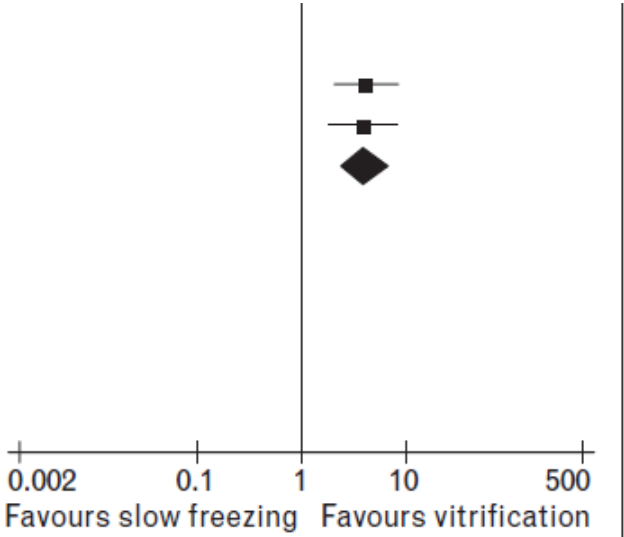
Cryopreservation of blastocysts by vitrification vs. Slow freezing?? Which one is better?

Cryosurvival

Study or Subgroup	Vitrification Events	Vitrification Total	Slow freezing Events	Slow freezing Total	Weight	Odds Ratio M-H, Random, 95% CI
2.1.2 Blastocyst stage						
Bernal et al., 2008	159	171	110	145	17.8%	4.22 [2.10, 8.48]
Huang et al., 2005	68	81	41	72	17.6%	3.95 [1.86, 8.41]
Subtotal (95% CI)		252		217	35.4%	4.09 [2.45, 6.84]
Total events	227		151			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.01$, df = 1 ($P = 0.90$); $I^2 = 0\%$

Test for overall effect: Z = 5.39 ($P < 0.00001$)



Only Bernal compared CPR, no sig. diff.

Efstratios et al.,Cur.Op.Obs&Gynec. 2009

Table 3. Review for the outcome of several studies on vitrification of human blastocysts.

Reference	*Cryo-protectant*	*Cryo-carrier*	*No. of vitrified blastocysts*	*Survival rate (%)*	*Pregnancy rate (%)*	*Implantation rate (%)/ comment*
Liebermann and Tucker, 2006	EG	FDP	254	Day 5 95.9; day 6 97.5	Day 5 48.7, day 6 42.8	Day 5 33; day 6 25
Utsunomiya *et al.*, 2006	–	Straw	(142 cycles)	87 (protocol 1), 89.6 (protocol 2), 89.8 (protocol 3)	35.0 (protocol 1), 32.0 (protocol 2), 11.1 (protocol 3)	25.9 (protocol 1), 22.8 (protocol 2), 9.4 (protocol 3)
Kuwayama *et al.*, 2005	DMSO/EG	Cryotop	6484	90	53	Cryotop was superior
Zech *et al.*, 2005	DMSO/EG	Hemi-straw	177	64–82	21–35	SR increased with intact ZP
Takahashi *et al.*, 2005	DMSO/EG/S	Cryoloop	1129	85.7	44	Congenital defects 1.4%
Huang CC *et al.*, 2005	DMSO/S/ EG/ HSA	Cryoloop	249	77.1	53.8	NA
Stehlik *et al.*, 2005	EG-based	Cryotop	41	100	50	NA
Hiraoka *et al.*, 2004	DMSO/EG	Cryotop	49	98	50	33
Vanderzwalen *et al.*, 2003	DMSO/EG	Hemi-straw	281	60	27 (ongoing)	AH more favourable implantation rate
Mukaida *et al.*, 2003a	EG-based	Cryoloop	444	79	36	NA
Mukaida *et al.*, 2003b	EG-based	Cryoloop	725	80.4	37	87; day 5 survival rate is higher
Cho *et al.*, 2002	EG-based	EM	293	50–82	34.1	Six step dilution of cryoprotectant was better
Reed *et al.*, 2002	EG/DMSO	Cryoloop	15	100	25	15.4
Vanderzwahln *et al.*, 2002	EG/Ficoll/S	Straws-direct plunge	167	20.3–58.5	4.5–20.5	Puncturing of blastocoele increased survival and pregnancy
Mukaida *et al.*, 2001	EG-based	Cryoloop	60	63	31.5	NA

>10.000 blasts. vitrified

Youssry et al.,RBM Online 2008

AH = assisted hatching, DMSO = dimethylsulphoxide, EG = ethylene glycol, EM = electron microscope grids, FDP = flexipet denuding pipette, HAS = human serum albumin S = sucrose, SR = survival rate, NA = not available, ZP = zona pellucida.

Outcome of vitrified blastocysts: 1278 cycles; donor &infertile patients

	D5		D6	
	n (%)	95% CI (%)	n (%)	95% CI (%)
No. of warming cycles	675		603	
No. of warmed embryos	1,079		952	
Age (y)	38.9 ± 5.5[b]	(38.5–39.3)	38.6 ± 5.5[b]	(38.2–39.0)
Survival rate	1,033 (95.7)[a]	(94.5–96.9)	929 (97.6)[b]	(96.9–98.6)
No. of embryo transfers (%)	649 (96.2)[b]	(94.7–97.6)	589 (97.6)[b]	(96.4–98.8)
No. of embryos replaced (mean ± SD)	1,008 (1.5 ± 0.6)[b]	(1.5–1.6)	919 (1.5 ± 0.6)[b]	(1.5–1.6)
Implantation rate	426 (42.3)[c]	(39.3–45.3)	390 (42.4)[c]	(39.2–45.6)
CPR/cycle	295 (43.7)	(40.0–47.4)	251 (41.6)	(37.7–45.5)
CPR/transfer	295 (45.5)	(41.7–49.3)	251 (42.6)	(38.6–46.6)
OPR/cycle	235 (34.9)	(31.3–38.5)	178 (29.5)	(25.8–33.1)
OPR/transfer	235 (36.2)	(32.5–39.9)	178 (30.7)	(27.0–34.4)
Miscarriage rate	56 (18.9)	(14.4–23.4)	73 (29)	(23.4–34.6)
DR/cycle	235 (34.8)	(31.2–38.4)	178 (29.5)	(25.9–33.1)
LBR/cycle	274 (40.6)[a,b]	(36.9–44.3)	196 (32.5)[a,c]	(28.8–36.2)

Note: CPR = clinical pregnancy rate; OPR = ongoing pregnancy rate; DR = delivery rate; LBR = live birth rate.
[a,b,c] Different superscripts in the same row indicate statistical difference ($P < .05$).

Van Landuyt et al.,HR 2011, 759 IVF/ICSI cycles,
Survival day 5: 79.3%,day 6:70.1%
SET OPR:14.2%, DET OPR: 20.5%,IR day 5:14.3%, day 6:13.7%

Cobo et al., F&S 2012

Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study

Z. Li[1,2], Y.A. Wang[1,3], W. Ledger[1], D.H. Edgar[4], and E.A. Sullivan[1,2,*]

SUMMARY ANSWER: Compared with slow frozen blastocysts, vitrified blastocysts resulted in significantly higher clinical pregnancy and live delivery rates with similar perinatal outcomes at population level.

STUDY DESIGN, SIZE, DURATION: A population-based cohort of autologous fresh and initiated thaw cycles (a cycle where embryos were thawed with intention to transfer) performed between January 2009 and December 2011 in Australia and New Zealand was evaluated retrospectively. A total of 46 890 fresh blastocyst transfer cycles, 12 852 initiated slow frozen blastocyst thaw cycles and 20 887 initiated vitrified blastocyst warming cycles were included in the data analysis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Pairwise comparisons were made between the vitrified blastocyst group and slow frozen or fresh blastocyst group. A Chi-square test was used for categorical variables and *t*-test was used for continuous variables. Cox regression was used to examine the pregnancy outcomes (clinical pregnancy rate, miscarriage rate and live delivery rate) and perinatal outcomes (preterm delivery, low birthweight births, small for gestational age (SGA) births, large for gestational age (LGA) births and perinatal mortality) following transfer of fresh, slow frozen and vitrified blastocysts.

MAIN RESULTS AND THE ROLE OF CHANCE: The 46 890 fresh blastocyst transfers, 11 644 slow frozen blastocyst transfers and 19 978 vitrified blastocyst transfers resulted in 16 845, 2766 and 6537 clinical pregnancies, which led to 13 049, 2065 and 4955 live deliveries, respectively. Compared with slow frozen blastocyst transfer cycles, vitrified blastocyst transfer cycles resulted in a significantly higher clinical pregnancy rate (adjusted relative risk (ARR): 1.47, 95% confidence intervals (CI): 1.39–1.55) and live delivery rate (ARR: 1.41, 95% CI: 1.34–1.49). Compared with singletons born after transfer of fresh blastocysts, singletons born after transfer of vitrified blastocysts were at 14% less risk of being born preterm (ARR: 0.86, 95% CI: 0.77–0.96), 33% less risk of being low birthweight (ARR: 0.67, 95% CI: 0.58–0.78) and 40% less risk of being SGA (ARR: 0.60, 95% CI: 0.53–0.68).

Human Reproduction, Vol.29, No.12 pp. 2794–2801, 2014

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

Alpha Scientists in Reproductive Medicine [1,*]

Reproductive BioMedicine Online (2012) 25, 146–167

Table 4 Blastocyst key performance indicator values.

KPI			*Competence*	*Benchmark*
B1	Survival rate	Freezing	70%	85%
		Vitrification	80%	95%
B2	Transfer rate	Freezing	70%	85%
		Vitrification	80%	95%
B3	Implantation rate		≤10% (relative) lower than that for the comparable population of fresh embryos at the centre	The same as for the comparable population of fresh embryos at the centre

The KPI values are calculated as the proportion of all thawed/warmed blastocysts.

ET rate: proportion of thawed/warmed blasts. that are sufficient quality to transfer

Neonatal Outcome of Vitrified Cleavage Stage Embryos

TABLE 1

Clinical parameters of fresh and vitrified day 3 embryos transfers.

Parameters	Fresh cycle	Vitrification
Patients' age (y)	32.11 ± 4.61	31.44 ± 4.75
No. of total cycles	604	312
No. of cycles transferred	598	285
No. of embryos transferred	1,576	817
Mean no. of embryos transferred	2.63	2.87
Implantation rate (%)	371 (23.54)	148 (18.11)[a]
No. of clinical pregnancies (% per embryo transfer)	251 (41.97)	105 (36.84)
No. of deliveries (% per embryo transfer)	166 (27.75)	69 (24.21)
No. of miscarriages (% per embryo transfer)	48 (8.02)	22 (7.71)
No. of ongoing pregnancies (% per embryo transfer)	28 (11.15)	11 (10.47)
No. lost follow-up (% per embryo transfer)	9	3

Note: Values are expressed as mean ± SD.

[a] $P < .05$, when compared to fresh cycle.

Rama Raju. Neonatal outcome of vitrified embryos. Fertil Steril 2008.

Rama Raju et al. F&S 2009

No.sig. dif. for neonatal parameters:

Mean gestational age, birth weights for singleton & MPR,
PR induced complications,
Incidence of birth defects (major & minor malformations)

Perinatal &neonatal outcomes of 494 babies from 972 vitrified day 3 ET

Comparison of obstetrical and neonatal outcomes between vitrified and fresh ETs.

	Singleton gestation		Multiple gestation	
Parameter	**Fresh**	**Vitrification**	**Fresh**	**Vitrification**
No. of vaginal deliveries (%)	76 (18.05)	34 (13.28)	21 (10.88)	11 (9.24)
No. of cesarean sections (%)	345 (81.95)	222 (86.72)	172 (89.12)	108 (90.76)
Mean gestational age, wk	38.85 ± 1.47	39.02 ± 1.67	36.67 ± 2.01	36.77 ± 1.56
No. of preterm deliveries (<37 wk) (%)	29 (6.89)	17 (6.64)	89 (46.11)	62 (52.10)
No. of very preterm deliveries (<32 wk) (%)	1 (0.24)	3 (1.17)	6 (3.11)	1 (0.84)
No. of cases of antenatal bleeding (%)	113 (26.84)	60 (23.44)	62 (32.12)	34 (28.57)
No. of cases of gestational diabetes (%)	14 (3.33)	6 (2.34)	12 (6.22)	5 (4.20)
No. of cases of pregnancy-induced hypertension (%)	16 (3.80)	10 (3.91)	17 (8.81)	19 (15.97)
Live birth	421	256	386	238
Male	197 (46.79)	126 (49.22)	178 (46.11)	107 (44.96)
Female	224 (53.21)	130 (50.78)	208 (53.89)	131 (55.04)
Mean birth weight, g	3,216.87 ± 451.30	3,337.44 ± 505.60[a]	2,435.23 ± 435.74	2,556.45 ± 432.91[a]
Mean Apgar score				
1 min	9.00 ± 0.07	8.91 ± 0.45[a]	8.91 ± 0.33	8.90 ± 0.39
5 min	9.91 ± 0.30	9.85 ± 0.47	9.36 ± 0.52	9.34 ± 0.56
10 min	9.92 ± 0.27	9.89 ± 0.37	9.45 ± 0.57	9.47 ± 0.56
Birth weight <1,500 g (%) **	0	2 (0.78)	10 (2.59)	0[b]
Birth weight 1,500–2,499 g (%)	24 (5.70)	10 (3.91)	186 (48.19)	99 (41.60)[b]
Birth weight 2,500–4,000 g (%)	371 (88.12)	221 (86.33)	190 (49.22)	138 (57.98)[b]
Birth weight ≥4,000 g (%)	26 (6.18)	23 (8.98)	0	1 (0.42)[b]
Neonatal intensive care unit admission (%)	59 (14.01)	30 (11.72)	169 (43.78)	71 (30.08)[c]
Neonatal death	0	4 (all for asphyxia)	5 (3 for asphyxia, 1 for congenital heart disease, 1 for neonatal necrotizing enterocolitis)	2 (1 for spinal bifida, 1 for lower limb malformation)
Neonatal complications				
Pathological jaundice	20 (2 for HDN)	11	13	11 (1 for HDN)
Respiratory problems	17	10	21	4
Low birth weight	18	5	129	56
Neonatal septicemia	0	1 (complicating pneumonia)	0	0
Others	4 (1 for hypoxic-ischemic encephalopathy, 1 for diarrhea, 1 for peptic ulcer, 1 for intracranial haemorrhage)	0	1 (fungus infection)	1 (fever)

Note: Values are expressed as mean ± SD. HDN = hemolytic disease of newborn.

Shi et al., F&S 2012

Neonatal outcome and birth defects in 6623 singletons born following minimal ovarian stimulation and vitrified versus fresh SET
Kato O et al., Eur J Obst.Gyn. Reprod Biol. 2012

- Vitrification of embryos/blastocysts did not increase the incidence of adverse neonatal outcomes or birth defects following SET, no sig. diff. in LGA

Obstetric and Neonatal outcomes after transfer of vitrified early cleavage stage embryos.
Liu SY et al., HR 2013

- No adverse effect on neonatal outcome . Birthweight was higher in the
- vitrified group versus slow or fresh ET implying an improved perinatal outcome

Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique?

A. Pinborg[1,*], A.A. Henningsen[2], A. Loft[2], S.S. Malchau[2], J. Forman[3], and A. Nyboe Andersen[2]

STUDY DESIGN, SIZE, DURATION: The national register–based controlled cohort study involves two populations of FET singletons. The first population (A: total FET cohort) consisted of all FET singletons ($n = 896$) compared with singletons born after Fresh embryo transfer (Fresh) ($n = 9480$) and also with that born after natural conception (NC: $n = 4510$) in Denmark from 1997 to 2006. The second population (B: Sibling FET cohort) included all sibling pairs, where one singleton was born after FET and the consecutive sibling born after Fresh embryo transfer or vice versa from 1994 to 2008 ($n = 666$). The sibling cohort included $n = 550$ children with the sibling combination first child Fresh/second child FET and $n = 116$ children with the combination first child FET/second child Fresh.

SUMMARY ANSWER: Singletons after FET have an increased risk of being born LGA. This cannot solely be explained by intrinsic maternal factors as it was also observed in sibling pairs, where the sibling conceived after FET had an increased risk of LGA compared with the sibling born after Fresh embryo transfer.

LIMITATIONS, REASONS FOR CAUTION: Adjustment for body mass index as a possible confounder was not possible. The size of the FET/Fresh sibling cohort was limited; however, the complete sibling cohort was sufficiently powered to explore the risk of LGA. A bias is very unlikely as data coding was based on national registers.

Age, parity, child sex, year of birth, birth order

- 1st. report 2008 from Australia by Shih, than Pelkonen 2010 and Sazonova 2012 reported LGA in FET singletons . ALL in slow-frozen embryos..

- 3 studies for vitrified embryos- not included in the meta-analysis

1. Kato 2012 that showed no diff. for embryos and blastocysts
2. Shi 2012 showed in a small population that mean birthweight is higher for vit. day 3 emb. BUT no risk calculations for LGA
3. Liu 2013 showed sig. higher birthweight with vit. compared to slow f. &fresh

- How does slow freezing or vitrification affect early embryo and plasental development and intrauterine growth environment should be further explored?

Human Reproduction, Vol.29, No.3 pp. 618–627, 2014

Perinatal outcome of blastocyst transfer with vitrification using cryoloop: a 4-year follow-up study

	Fresh blastocyst transfer	%	Vitrified blastocyst transfer	%
Live born	205		147	
Male	120	58.5[a]	74	50.3
Female	85	41.5[a]	73	49.7
Vaginal delivery	70	45.8	44	40.7
Cesarean section	83	54.2	64	59.3
Mean gestational age (wk)	37.9 ± 2.5		38.1 ± 2.8	
Preterm (<37 wk)	19	12.4	20	18.5
Mean birth weight (g)	2,593 ± 629		2,601 ± 709	
<1,500 g	9		8	
1,500–2,500 g	66		56	
>2,500 g	130		83	
Twins	40	26.1	30	27.8
Triplets	6	3.9	4	5.1

[a] $P<.05$.

Prevalence of major and minor birth defects in infants.

	Fresh blastocyst transfer	Vitrified blastocyst transfer
Major defects	1 (21 trisomy, deceased)	1 (Treacher-Collins syndrome[a])
Minor defects	3 (anal atresia, PDA, defect of 4th lumbar spine)	1 (PDA)
Total (%)	4 (2.0%)	2 (1.4%)

[a] Mandibulofacial dysostosis.

Takahashi et al.,F&S 2005

Liebermann F&S 2006, Wennerholm 2009, Wikland 2010 ,Kato 2012, also reported no adverse effect

Obstetric outcomes after transfer of vitrified blastocysts

M. Wikland[1,*], T. Hardarson[1], T. Hillensjö[1], C. Westin[1], G. Westlander[1], M. Wood[1], and U.B. Wennerholm[2]

METHODS: All children born after transfer of vitrified blastocysts ($n = 106$), fresh blastocysts ($n = 207$) and slow-frozen early cleavage stage embryos ($n = 206$) during the period January 2006 to May 2008 at Fertility Center Scandinavia were included. Data on obstetric and neonatal outcomes were obtained from medical records from the antenatal and delivery clinics.

RESULTS: For singletons, there were no significant differences between the groups in gestational age, mortality or birth defects. After adjustment for parity and BMI, birthweight was significantly higher in singletons born after transfer of vitrified blastocysts as compared with after transfer of fresh blastocysts (median 3560 versus 3510 g, $P = 0.0311$). More singletons born after transfer of fresh blastocysts were small for gestational age compared with singletons born after transfer of vitrified blastocysts (12.1 versus 3.0%, $P = 0.0085$). A higher rate of major post-partum haemorrhage was observed in the vitrified blastocyst group as compared with the other two groups (25.0 versus 6.0 and 7.5%).

CONCLUSIONS: No adverse neonatal outcomes were observed in children born after transfer of vitrified, as compared with fresh blastocysts or after transfer of slow-frozen early cleavage stage embryos.

Human Reproduction, Vol.25, No.7 pp. 1699–1707, 2010

Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers

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Singleton neonatal outcomes for fresh and vitrified-warmed blastocyst transfers.

	Fresh	Vitrified	*P* value
Maternal age[a]	34.8 ± 3.6	34.8 ± 4.0	
Median (IQR)	35.1 (32.1–37.8)	35.0 (32.1–37.8)	.858
Gestational age[a] (wk)	39.0 ± 2.2	39.3 ± 2.0	
Median (IQR)	39.4 (38.4–40.3)	39.4 (38.8–40.6)	.047
Preterm birth rate	44/507 (8.7%)	13/229 (5.7%)	.158
Live-birth weight[a] (g)	3,296 ± 563	3,441 ± 554	
Median (IQR)	3,300 (3,000–3,660)	3,465 (3,173–3,778)	<.001
Low-birth-weight rate	28/507 (5.5%)	10/229 (4.4%)	.512
Neonatal death rate	1/507 (0.2%)	1/229 (0.4%)	1

Note: Data is singleton births from combined grade I and grade II blastocyst transfers. There were 5 fetal heart positive pregnancies (from a total of 859) for whom birth outcome data were not available. IQR = interquartile range.

[a] Mean ± standard deviation.

*** 145gr. heavier**

Retrospective study of 1209 patients: 1157 fresh vs. 645 FET, similar LBR, and improved neonatal outcomes

Fertility and Sterility® Vol. 101, No. 5, May 2014

Poor cryosurvival rates (~30%) and clinical outcome after conventional slow freezing of biopsied cleavage stage embryos (Joris et al., HR 1999, Magli et al., HR 1999)

Vitrification results with higher cryosurvival rates for biopsied human embryos

Table I. Survival rate of embryos post-freezing–thawing with different methods

Group	Method	No. of embryos frozen	No. of surviving embryos (%)[a]	No. of intact embryos (%)[b]	Total no. of blastomeres surviving (%)[c]
1	Standard	Non-biopsied 53	45 (85)	19 (36)	223/316 (71)
2	Standard	Biopsied 52	8 (16)	5 (10)	65/261 (25)
3	Modified freezing	Biopsied 52	39 (75)	21 (40)	175/278 (63)
4	Modified thawing	Biopsied 50	38 (76)	7 (14)	142/258 (55)
5	Vitrification	Biopsied 49	46 (94%)	39 (80%)	218/243 (90%)

[a] $P < 0.0005$ for 2 versus 1, for 2 versus all others; $P < 0.05$ for 5 versus 3 or 4; $P > 0.05$ for 1 versus 3, 4 or 5.
[b] $P < 0.005$ for 2 versus 1, $P < 0.001$ for 2 versus 3; $P > 0.05$ for 2 versus 4; $P < 0.0005$ for 2 versus 5; $P < 0.0005$ for 5 versus 1, 3 or 4; $P > 0.05$ for 1 versus 3.
[c] $P < 0.0005$ for 2 versus all others; $P < 0.0005$ for 5 versus 3, 4 and 1; $P < 0.05$ for 1 versus 3; $P < 0.0005$ for 1 versus 4; $P > 0.05$ for 3 versus 4.

Zheng et al.,HR 2005

- **Survival and IR of biopsied cleavage embs. and vit. at blastocyst stage is similar with non-biopsied counterparts. El-Toukhy HR 2009**
- **Higher survival and preg.for biopsied and vit. embryos compared to biopsied and slowly frozen Keskintepe JARG 2009**

Vitrification results with high cryosurvival rates for biopsied blastocysts

- Escriba et al., F&S 2008 similar cumulative OPR/OPU for PDG
- and non-PGD group with blastocyst vitrification
- Schoolcraft et al., F&S 2013 CGH of TE with vitrification facilitates e SET for infertile women with advanced maternal age
- Zhang et al.,F&S 2014 Blasts. can be rebiopsied &vitrified without diminishing IR, and LBR
- Reed et al. JARG 2014 Similar clinical outcome for biopsied and non-biopsied blasts. With large-volume vitrification
- Taylor et al.,RBM 2014 No dif. in clinical outcome for single or double vitrified biopsied blastocysts

Flexibility of re-cryopreserving cells by vitrification method

- It's presumed that refrozen & thawed embryos using conventional methods results with detrimental cryoinjury

- Chang C . RBM Online 2008 Two successful pregnancies obtained following oocyte vitrification and embryo re-vitrification.
- Kumasako et al., F&S 2009 The efficacy of the transfer of twice frozen thawed embryos with the vitrification method
- Peng et al.,RBM Online 2011 Live birth after transfer of a twice vitrified warmed blastocyst that had undergone trophoectoderm biopsy
- James et al., RBM Online 2012 Vitrification of human embryos previously cryostored by either slow freezing or vitrification results in high pregnancy rates
- Cobo et al., F&S 2013 Outcome of cryotransfer of embryos developed from vitrificed oocytes: double vitrification has no impact on delivery rates
- Greco et al., Springerplus. 2015 Successful implantation and live birth of a healthy boy after triple biopsy and double vitrification of oocyte-embryo-blastocyst.

30 Eylül 2014 Sayı : 29135
Sağlık Bakanlığı
ÜREMEYE YARDIMCI TEDAVİ UYGULAMALARI VE ÜREMEYE YARDIMCI TEDAVİ MERKEZLERİ HAKKINDA YÖNETMELİK

- **BEŞİNCİ BÖLÜM**
- **Hizmet Sunumuna İlişkin Diğer Esaslar**
- **Üreme hücreleri ve gonad dokularının saklanma kriterleri**
- **MADDE 20**
- (5) Adaylardan fazla embriyo elde edilmesi durumunda eşlerden her ikisinin rızası alınarak embriyolar dondurulmak suretiyle saklanır. Saklama süresinin bir yılı aşması halinde her yıl embriyonun saklanması için çiftler mutlaka başvuruda bulunarak taleplerinin devam ettiğini ifade eden imzalı dilekçe vermelidir. Eşlerin birlikte talebi, eşlerden birinin ölümü veya boşanmanın hükmen sabit olması halinde ya da belirlenen süre son bulduğunda saklanan embriyolar müdürlükte kurulacak komisyon tarafından tutanak altına alınarak imha edilir.
- (6) Bu maddenin ikinci ve üçüncü fıkralarında belirtilen numuneler, merkezlerde en fazla beş yıl süreyle saklanır. Beş yıldan fazla saklanması Bakanlığın iznine tabidir. Saklanan numunelerin değerlendirmeleri, sayımları ve tekrar kullanılmasını engelleyecek şekilde imhası ilgili müdürlük bünyesinde kurulacak komisyon marifetiyle yapılır.
- (7) Merkezlerde saklanan dondurulmuş embriyo ve/veya gonad dokusu/hücresi,
- a) Embriyo için eşlerin birlikte, gonad dokusu/hücresi sahibinin ise bireysel olarak her iki merkeze yazılıbaşvuruda bulunması,
- b) Embriyo ve/veya gonad dokusu/hücresinin teslim edildiği ve teslim alındığına dair yazılı olarak müdürlüğe bildirimde bulunulması,
- c) Transferin tüm sorumluluğunun ve ücretinin talep edene ait olması,
- ç) Transfere ait teknik donanım ve altyapının transferin gerçekleştirileceği merkezce sağlanması,
- d) Transferin gerçekleştirileceği tankın transfer edilecek materyalin saklandığı merkez tarafından mühürlenmesi ve materyalin teslim alındığı merkez tarafından mührün kontrol edilerek kendileri tarafından açıldığının tutanak altına alınması,

halinde yurtiçindeki başka bir merkeze transfer edilebilir.

THANK YOU !!

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