Cryopreservation of Oocytes Indications

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OVERVIEW

- Clinical outcome for oocyte freezing; Why vitrification?

** Implications:

- Fertility preservation in cancer patients
- Fertility preservation for social reasons
- Ovum donation programs
- Minimization of ovarian hyperstimulation syndrome risk, oocyte accumulation in low responder patients
- Surplus oocyte cryostorage after COH when embryo freezing is not feasible
- Neonatal safety
- Future directions

Cobo & Diaz F&S 2011

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

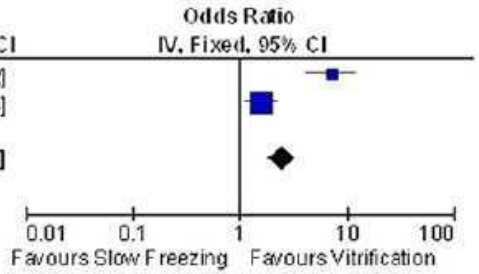
FIGURE 2

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

A Survival rate of Vitrification vs. Slow freezing. Fixed effects model

Study or Subgroup	Vitrification Events	Vitrification Total	Slow Freezing Events	Slow Freezing Total	Weight	Odds Ratio IV, Fixed, 95% CI
Cao 2009	268	292	75	123	29.7%	7.15 [4.11, 12.42]
Smith 2010	260	349	155	238	70.3%	1.56 [1.09, 2.24]
Total (95% CI)		641		361	100.0%	2.46 [1.82, 3.32]
Total events	528		230			

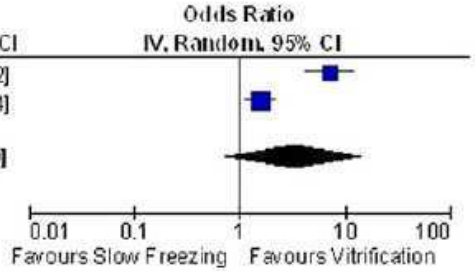
Heterogeneity: Chi² = 20.40, df = 1; I² = 95%
Test for overall effect: Z = 5.85



B Survival rate of Vitrification vs. Slow freezing. Random effects model

Study or Subgroup	Vitrification Events	Vitrification Total	Slow Freezing Events	Slow Freezing Total	Weight	Odds Ratio IV, Random, 95% CI
Cao 2009	268	292	75	123	49.0%	7.15 [4.11, 12.42]
Smith 2010	260	349	155	238	51.0%	1.56 [1.09, 2.24]
Total (95% CI)		641		361	100.0%	3.29 [0.74, 14.59]
Total events	528		230			

Heterogeneity: Tau² = 1.10; Chi² = 20.40, df = 1; I² = 95%
Test for overall effect: Z = 1.57



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

Cobo &Diaz F&S 2011

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

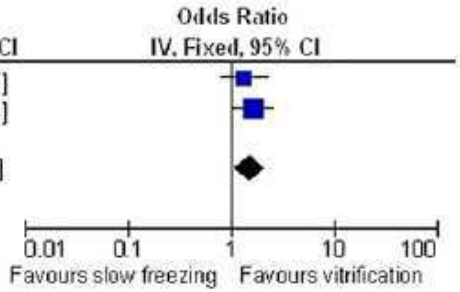
FIGURE 3

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

A Vitrification vs. Slow freezing. Fixed effects model

Study or Subgroup	Vitrification Events	Total	Slow Freezing Events	Total	Weight	Odds Ratio IV, Fixed, 95% CI
Cao 2009	182	268	46	75	41.0%	1.33 [0.78, 2.27]
Smith 2010	200	260	104	155	59.0%	1.63 [1.05, 2.54]
Total (95% CI)		528		230	100.0%	**1.50 [1.07, 2.11]**
Total events	382		150			

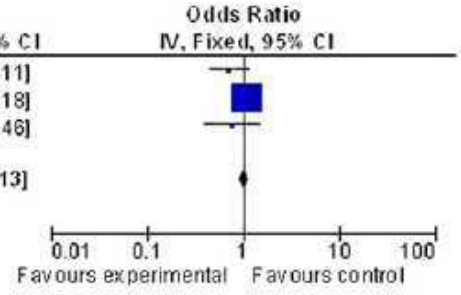
Heterogeneity: $Chi^2 = 0.33$, $df = 1$; $I^2 = 0\%$
Test for overall effect: $Z = 2.36$



B Vitrification vs. Fresh oocytes. Fixed effects model

Study or Subgroup	Vitrification Events	Total	Fresh Events	Total	Weight	Odds Ratio IV, Fixed, 95% CI
Cobo 2008	171	224	180	219	5.5%	0.70 [0.44, 1.11]
Cobo 2010	2256	3039	2334	3185	91.8%	1.05 [0.94, 1.18]
Rienzi 2010	95	120	100	120	2.8%	0.76 [0.40, 1.46]
Total (95% CI)		3383		3524	100.0%	**1.02 [0.91, 1.13]**
Total events	2522		2614			

Heterogeneity: $Chi^2 = 3.60$, $df = 2$; $I^2 = 44\%$
Test for overall effect: $Z = 0.33$



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

Cobo &Diaz F&S 2011

Review of literature for Oocyte Cryopreservation

Table 1: Results from different oocyte cryopreservation protocols: slow freezing (high-sucrose concentration) and vitrification.

	Vitrification (VIT)	Slow freezing (SF)	Survival	Fertilization	Pregnancy	Miscarriage	Egg donation program
Chen et al., 2005 [44]	—	Yes	75% (119)	67% (80)	33% (7)	0%	Partially
Li et al., 2005 [45]	—	Yes	90% (73/81)	82% (60/73)	47% (7/15)	28% (2/7)	Partially
Kuwayama et al., 2005 [46]	Yes	—	91% (58/64)	81% (52/64)	41% (12/29)	17% (2/12)	No
Borini et al., 2006 [47]	—	Yes	43,4% (306/705)	51,6% (158/306)	19,2% (14/73)	28,6% (4/14)	No
Barritt et al., 2007 [48]	—	Yes	86,1% (68/79)	89,7% (61/68)	75% (3/4)	NS	Yes
Lucena et al., 2006 [49]	Yes	—	96,7% (143)	87,2% (105)	56,5% (13)	NS	Yes
Antinori et al., 2007 [50]	Yes	—	99,4% (328/330)	92,9% (305/328)	32,5% (39/120)	20,5% (8/39)	No
Cobo et al., 2008 [51]	Yes	—	96,9% (224/231)	76,3% (171/224)	65,2% (15/23)	20% (3/15)	Yes
Parmegiani et al., 2008 [52]	—	Yes	75,1% (328/437)	80% (227/328)	19% (16/83)	31,2% (5/16)	No
Cao et al., 2009 [42]	Yes	Yes	SF 61% (75/123)	SF 61,3% (46/75)	ND	ND	No
			VIT 91,8% (268/292)	VIT 67,9% (182/268)	ND	ND	No
Smith et al., 2010 [53]	Yes	Yes	SF 65% (155/238)	SF 67% (104/155)	SF 13% (4/30)	SF 25% (1/4)	No
			VIT 75% (260/349)	VIT 77% (200/260)	VIT 38% (18/48)	VIT 18% (4/18)	No
Rienzi et al., 2010 [54]	Yes	—	97% (120/124)	79,2% (95/120)	30,8% (15/39)	20% (3/15)	No
Cobo et al., 2010 [55]	Yes	—	92,5% (3039)	73,3% (NS)	55,4% (148)	NS	Yes

NS = Data not reported.
ND = Data not calculated, not a study endpoint.

Revelli et al., Obst. &Gyn. Int 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 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		

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 <43 years of age requiring ICSI with >6 mature oocytes	Infertile patients <42 years of age requiring ICSI with >5 mature oocytes
No. patients	30 vitrification 30 fresh	295 vitrification 289 fresh	40 vitrification 40 fresh	31 vitrification 31 fresh
Mean age at retrieval	26	26	35	35
No. oocytes	231 vitrification 219 fresh	3286 vitrification 3185 fresh	124 vitrification 120 fresh	168 vitrification NA fresh
No. oocytes per retrieval	18.2	11	13	NA
Survival	96.9%	92.5%	96.8%	89.9%
Fertilization rate	76.3 vitrification 82.2 fresh	74% vitrification 73% fresh	79.2% vitrification 83.3% 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	2	2–3
Implantation rate	40.8% vitrification 100% fresh	39.9% vitrification 40.9% fresh	20.4% vitrification 21.7% fresh	17.1% vitrification NA fresh
CPR/transfer vitrification vs. fresh	60.8% (23 vitrification transfers) 100% (1 fresh transfer)	55.4% vitrification 55.6% fresh	38.5% vitrification 43.5% fresh	35.5% vitrification 13.3% fresh
CPR/oocyte thawed	6.1%	4.5%	12%	6.5%

Note: All used vitrification with Cryotop, 15% EG + 15% DMSO + 0.5M sucrose. CPR = clinical pregnancy rate.

Practice Committee. Oocyte cryopreservation. Fertil Steril 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

Majority of successful clinical trials performed with open carriers?
Cross contamination???

Storage of human oocytes in the vapor phase of nitrogen

Objective: To evaluate the effectiveness of long-term vapor-phase nitrogen storage of vitrified human oocytes as a strategy for preventing the risk of cross-contamination due to direct contact with the liquid nitrogen (LN).
Design: Prospective randomized study.
Setting: Private infertility center, IVI, Valencia.
Patient(s): Oocyte donors (n = 44) and recipients (n = 46).
Intervention(s): Vitrification by the Cryotop method. Storage of vitrified oocytes in a vapor-phase nitrogen storage freezer and a traditional LN storage tank. Donation of the surviving oocytes and evaluation of fertilization, embryo development, and clinical results.
Main Outcome Measure(s): Survival, fertilization, and cleavage rates. Embryo quality and clinical outcome.
Result(s): Survival was 95.3% (vapor-phase nitrogen) and 94.5% (LN). Fertilization rates (73.1% and 71.7%) or cleavage on day 2 (95.6% and 94.7%), day 3 (84.5% and 79.9%), and blastocyst formation (54.7% and 53.9%) were similar between vapor-phase nitrogen and LN. Implantation, clinical, and ongoing pregnancy rates were similar for vapor-phase nitrogen (40.5%, 58.1%, and 48.8%, respectively) and LN groups (33.7%, 53.3%, and 46.6%, respectively).
Conclusion(s): The vapor-phase nitrogen system permits the storage of oocytes vitrified, maintaining their potential to develop into competent embryos in a similar manner as those stored in a traditional LN freezer. This approach represents a practical alternative that prevents cross-contamination during the storage of vitrified samples. (Fertil Steril® 2010;94:1903–7. ©2010 by American Society for Reproductive Medicine.)

Cobo et al., F&S 2010

Viral screening of spent culture media and liquid nitrogen samples of oocytes and embryos from hepatitis B, hepatitis C, and human immunodeficiency virus chronically infected women undergoing in vitro fertilization cycles

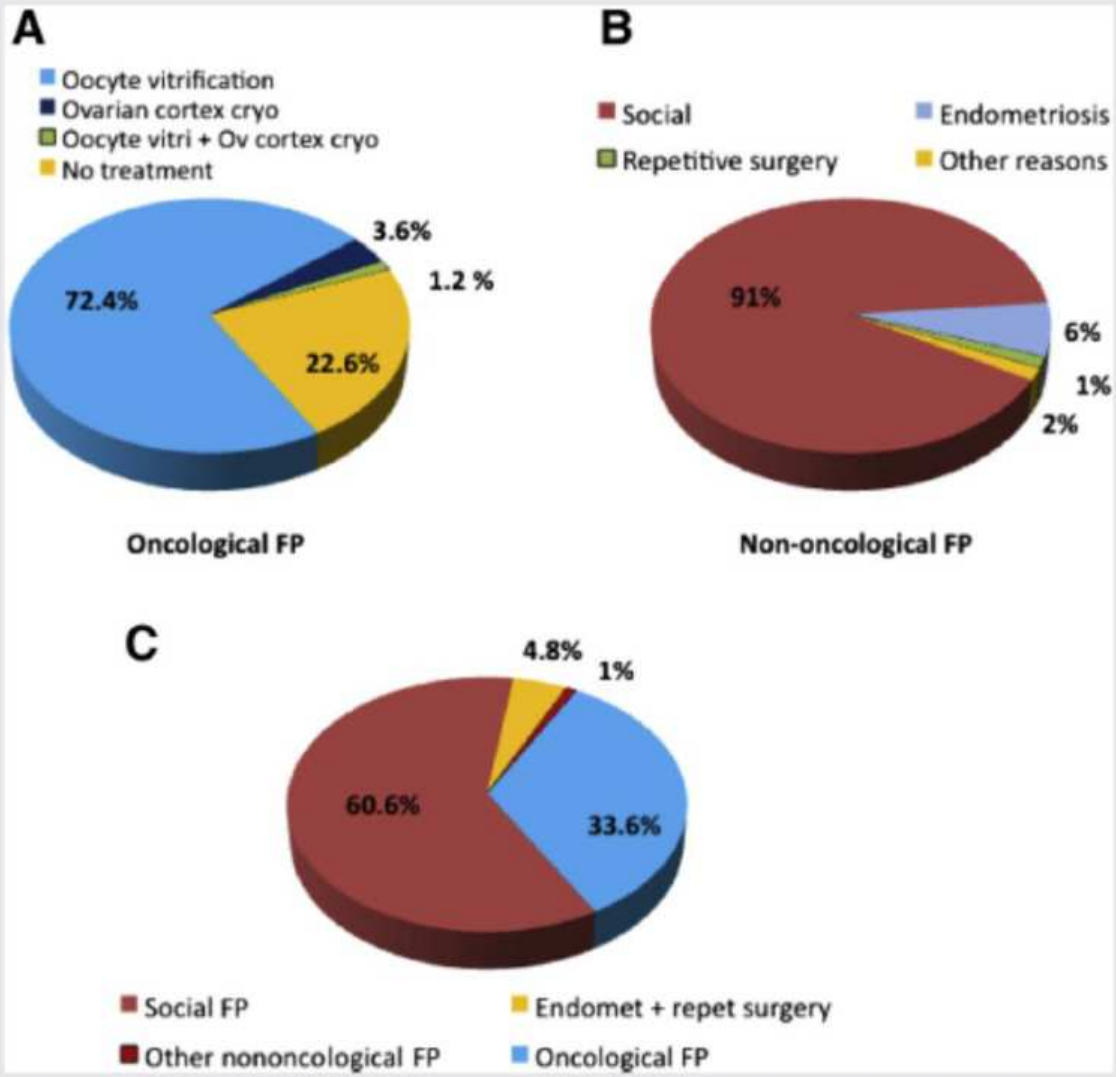
Objective: To assess the presence of viral RNA or DNA sequences in spent culture media used after ovum pickup (OPU) or embryo culture and in liquid nitrogen (LN) used for oocyte or embryo vitrification in patients seropositive for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) undergoing IVF cycles.
Design: Descriptive study.
Setting: Private university-affiliated IVF center.
Patient(s): Twenty-four women who underwent controlled ovarian stimulation for oocyte vitrification or IVF/ET. A total of 6, 11, and 6 patients were seropositive for HIV, HCV, and HBV, respectively, whereas 1 patient showed a coinfection with HCV and HBV. Seven patients presented positive blood viral load (HIV, n = 1; HBV, n = 1; HCV, n = 5). Sixty-three samples were analyzed: follicular fluid, n = 3; spent culture media, n = 33 (20 after OPU and 13 after embryo culture); and LN, n = 27 (14 and 10 after oocyte and embryo vitrification; and 3 LN storage tank samples).
Intervention(s): Ovum pickup, oocyte and/or embryo culture, and/or vitrification by the Cryotop open device. Reverse transcription–polymerase chain reaction analysis was performed for viral screening.
Main Outcome Measure(s): Detection of viral sequences of HIV, HCV, and HVB.
Result(s): All the samples analyzed tested negative for the detection of viral RNA or DNA sequences.
Conclusion(s): We have not detected viral sequences after culture and vitrification of oocytes/embryos from HIV-, HBV-, and HCV-seropositive patients. These findings represent good evidence of the lack of risk of cross-contamination among seropositive patients, even using an open device for vitrification. (Fertil Steril® 2012;97:74–8. ©2012 by American Society for Reproductive Medicine.)

Cobo et al., F&S 2012

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



(A) Fertility preservation (FP) options distribution of the oncological patients. (B) Distribution of nononcological patients according to their indication to cryopreserve (cryo) oocytes. (C) Indications of all the FP cases from our program. Endomet = endometriosis; Ov = ovarian; repet = repeat.

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 preserved 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 (120)
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 (EMG)	Vitrification (Cryotop)
Age at FP, y	27	26	36	22	31
No. of cryopreserved oocytes	13	7	16	7	4
Storage time (y)	6	4	2	9	2
Twin or single pregnancy	Single[a]	Twin	Twin	Single	Single
No. of live births	1	2	2	1	1
Weeks of gestation	37	38	34	35 + 3 d	39
Weight of baby, g	3,062	2,100 and 2,400	1,650 and 1,830	2,410	3,440
Sex of baby	Male	Females	Males	Male	Male

Note: EMG = electron microscope grids; FP = fertility preservation; OTC-SF = ovarian tissue cryopreservation; OV = oocyte vitrification.
[a] Gestational carrier.

Cobo. Oocyte vitrification for fertility preservation. Fertil Steril 2013.

Slow freezing no.of live birth 3
Vitrification 4

Cobo et al., F&S 2013

Social Oocyte freezing??

- Social, educational and financial pressures offen lead woman to delay starting a family until their late 30s, by which time the chance of success is compromised by low fecundity rates and an increased risk of miscarriage if they become pregnant. (Lockwood RBM Online 2011)

- Stoop et al., HR 2011: A survey on the intentions and attitudes towards oocyte cryopreservation for non-medical reasons among women of reproductive age: Significant proportion of young women (>51.8%) would consider safeguarding their reproductive potential or at least open to the idea of social oocyte freezing

- ESHRE Task force on Ethics and law. HR 2012 Oocyte cryopreservation for age-related fertility loss

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.**

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	513 (1.74 ± 0.7)	498 (1.72 ± 0.7)
Number of cycles with embryo 're-vitrification'/cryopreservation	196 (66.7)	216 (74.7)*
Mean number of re-vitrified or cryopreserved embryos	592 (2.0 ± 2.1)	743 (2.5 ± 2.3)*
Implantation rate	205 (39.9)	204 (40.9)
Positive hCG test/cycle	165 (55.9)	159 (55.0)
Clinical pregnancy rate/cycle	148 (50.2)	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 embyos (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

Oocyte cryopreservation for donor egg banking

Table 1 2008–2009 (24 month) outcome data using vitrified donor oocytes in IVF treatment for recipients in two IVF centres.

Outcome	IVI	RBA
Donation cycles	1051	168
Recipient cycles	919	322
Age (years)	41.2 ± 4.3	41.1 ± 4.9
Total oocytes warmed (per recipient)	12,786 (12.9 ± 4.0)	2001 (6.2 ± 1.9)
Total oocytes for ICSI	11,949 (11.4 ± 3.4)	1750 (5.4 ± 1.7)
Two-pronuclei ICSI fertilization rate	8920 (74.7)	1494 (85.4)
Good-quality embryos on day 3 (per inseminated oocyte)[a]	5366/11,949 (44.9)	979/1750 (55.9)
Good-quality embryos on day 5 (per embryo subjected to extended culture)[a]	1427/3568 (40.0)	582/1185 (49.1)
Implantation rate	655/1655 (39.6)	255/577 (44.2)
Embryos cryopreserved	1915 (1.8 ± 2.0)	414 (1.3 ± 1.5)
Clinical pregnancies (per transfer)[b]	502 (55.4)	182 (56.5)
Infants born[c]	343 (180 female; 163 male)	146 (64 female; 82 male)

Values are *n*, mean ± standard deviation, *n* (mean ± standard deviation), *n* (%) or *n*/total (%). ICSI = intracytoplasmic sperm injection, IVI = Instituto Valenciano de Infertilidad; RBA = Reproductive Biology Associates.

[a] IVI and RBA embryo scoring systems are different, thus it may not be possible to directly compare these numbers.

[b] Additionally, 25 (IVI) and 21 (RBA) more clinical pregnancies from subsequent embryo cryotransfer were obtained.

[c] Additionally, 10 infants born from subsequent embryo cryotransfer at IVI (six female and four male) and 17 infants at RBA (nine female and eight male). There is no data on all infants born during this period. Four newborns at RBA had birth defects.

Similar clinical efficiencies of 2 different ART programmes, IVI:1donor/1 receipient, RBA:1 donor/several rec.

Cobo et al.,RBM Online 2011

Embryo development of fresh vs. vitrified MII after ICSI (in non-donor cycles)

	Fresh ICSI	Vitrified/Warmed ICSI (%)	P
Fertilization (2PN) per sibling oocyte	100/120 (83.3)[b]	95/124 (76.6)[a]	0.20
Fertilization (2PN) per injected oocyte	100/120 (83.3)[b]	95/120 (79.2)[b]	0.50
Normal 2PN morphology	96/100 (96.0)[c]	86/95 (90.5)[c]	0.16
1PN oocytes	3/120 (2.5)[b]	6/120 (5.0)[b]	0.50
3PN	1/120 (0.83)[b]	2/120 (1.66)[b]	1
Degenerated oocytes post-ICSI	1/120 (0.83)[b]	4/120 (3.34)[b]	0.37
Day 2 embryo development	100/100 (100)[c]	93/95 (97.9)[c]	0.24
Excellent quality embryos	52/100 (52.0)[d]	49/95 (51.6)[d]	0.90
Good quality embryos	38/100 (38.0)[d]	41/95 (43.2)[d]	0.47
Fair/poor quality embryos	10/100 (10.0)[d]	3/95 (3.16)[d]	0.10

[a]Percentages, expressed per warmed oocyte.
[b]Percentages, expressed per inseminated oocyte.
[c]Percentages, expressed per 2PN fertilized oocyte.
[d]Percentages, expressed per cleaved oocyte.

Prospective-randomized sibling oocytes

	Patients included (*N* = 40)
Number of warmed oocytes (mean ± SD)	3.1 ± 0.30
Number of embryos transferred (mean ± SD)	2.3 ± 0.88
Number of embryo transfer performed (%)	39/40 (97.5)
Clinical pregnancy rate per cycle (%)	15/40 (37.5)
Clinical pregnancy rate per transfer (%)	15/39 (38.5)
Ongoing pregnancy rate per cycle (%)	12/40 (30.0)
Ongoing pregnancy rate per transfer (%)	12/39 (30.8)
Implantation rate (%)	19/93 (20.4)
Ongoing implantation rate (%)	16/93 (17.2)

Results of cryo cycles after 1 IF of fresh ICSI

Patients >42, <6MII, ICSI with ejeculated sperm,

Rienzi et al.,HR 2010

Cumulative OPR with Vitrification in all ICSI cycles

	Overall	≤34 years	35–37 years	38–40 years	41–43 years
Fresh cycles: clinical outcomes					
No. of cycles	182	72	48	41	21
No. of ET	172/182 (94.5)	66/72 (91.6)	46/48 (95.8)	40/41 (97.6)	20/21 (95.2)
Clinical pregnancy rate per cycle	77/182 (42.3)[a]	32/72 (44.4)	22/48 (45.8)	18/41 (43.9)	5/21 (23.8)
Clinical pregnancy rate per ET	77/172 (44.8)[b]	32/66 (48.5)	22/46 (47.8)	18/40 (45.0)	5/20 (25.0)
Implantation rate	101/435 (23.2)[c]	46/153 (30.0)	29/116 (25.0)[f]	21/112 (18.7)	5/54 (9.2)
Abortion rate	9/77 (11.7)	3/32 (9.4)	2/22 (9.0)	3/18 (16.7)	1/5 (20.0)
Ongoing pregnancy rate per fresh cycle	68/182 (37.4)[d]	29/72 (40.3)	20/48 (41.7)[g]	15/41 (36.6)	4/21 (19.0)
Ongoing implantation rate	90/435 (20.7)[e]	42/153 (27.4)	26/116 (22.4)[h]	18/112 (16.1)	4/54 (7.4)
Warmed cycles: clinical outcomes					
No. of cycles	115	37	30	30	18
No. of ET	111 (96.5)	35/37 (94.6)	29/30 (96.7)	30/30 (100)	17/18 (94.4)
Clinical pregnancy rate per cycle	35/115 (30.4)[a]	17/37 (45.9)	7/30 (23.3)	7/30 (23.3)	4/18 (22.2)
Clinical pregnancy rate per ET	35/111 (31.5)[b]	17/35 (48.6)	7/29 (24.1)	7/30 (23.3)	4/18 (22.2)
Implantation rate	43/266 (16.1)[c]	21/77 (27.3)	8/73 (10.9)[f]	9/75 (12.0)	5/41 (12.2)
Abortion rate	6/35 (17.1)	1/17 (5.9)	3/7 (42.8)	1/7 (14.3)	1/4 (25.0)
Ongoing pregnancy rate per warmed cycle	29/115 (25.2)[d]	16/37 (43.2)	4/30 (13.3)[g]	6/30 (20.0)	3/18 (16.6)
Ongoing implantation rate	35/266 (13.2)[e]	19/77 (24.7)	5/73 (6.8)[h]	8/75 (10.7)	3/41 (7.3)

Data are expressed as absolute and percentage frequency. ET, embryo transfer.
[a,b,c,d,e,f,g,h] $p < 0.05$.

Cumulative OPR

	Overall	≤34 years	35–37 years	38–40 years	41–43 years
Fresh cycle	68/182 (37.4%)	29/72 (40.3%)	20/48 (41.7%)	15/41 (36.6%)	4/21 (19.0%)
(95% CI)	(31.2–45.1)	(29.7–51.9)	(28.8–55.8)	(23.6–52.0)	(7.8–40.3)
I warming cycle	94/182 (51.6%)	45/72 (62.5%)	23/48 (47.9%)	20/41 (48.8%)	6/21(28.6%)
(95% CI)	(44.4–58.8)	(50.9–72.8)	(34.4–61.7)	(34.2–63.6)	(13.9–50.2)
II warming cycle	97/182 (53.3%)	45/72 (62.5%)[a]	24/48 (50.0%)	21/41 (51.2%)	7/21 (33.3%)[a]
(95% CI)	(40.0–60.0)	(50.9–72.8)	(36.3–63.6)	(36.4–65.8)	(17.2–54.9)

Data are expressed as absolute, percentage frequency and 95% CI.
[a] $P = 0.006$.

Pros.longitudinal cohort study
Maternal age is the only charecteristic influencing the reproductive outcome

Ubaldi& Rienzi HR 2010

Delivery rates after oocyte vitrification: Multicentric study

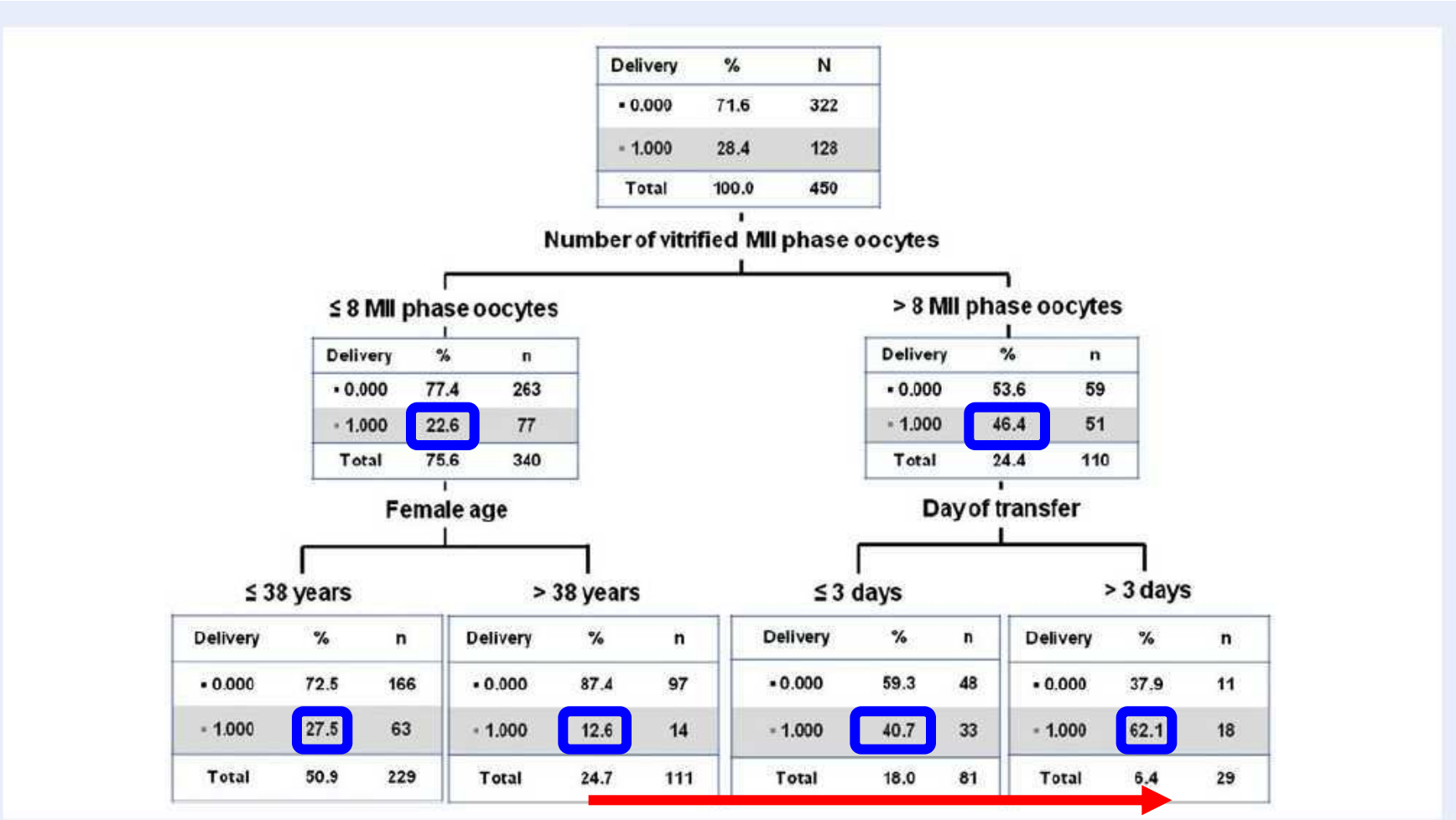


Figure 1 Decision-making model based on recursive partitioning analysis (per patient basis; black squares, 0 = delivery not obtained; grey squares, 1 = delivery obtained).

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

Rienzi et al., HR 2012

Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis

Characteristics and success rates of the studies from which IPD were available and used for meta-analysis.

Age (y)	Country	First author/year (reference)	Study type	Method	n	No. thawed oocytes	No. thaw cycles	SR (%)	FR (%)	IR (%)	CP/T (%)	LB/T (%)
31.7 ± 4.7	Taiwan	Chen/2005 (17)	Prospective	SF	21	159	21	76.1	66.1	10.7	33.3	33.3
31.7 ± 4.1	United States	Boldt/2006 (18)	Retrospective	SF	82	556	87	55.4	65.4	12.6	26.5	19.1
32.0 ± 3.6	United States	Boldt/2006[a]		VF	25	168	28	78.0	76.7	11.5	29.2	20.8
32.3 ± 3.6	Hungary	Konc/2008 (19)	Retrospective	SF	54	215	64	80.0	84.3	11.0	20.3	15.6
35.7 ± 5.7	Italy	Albani/2008 (20)	Retrospective	SF	949	7,584	1,280	58.1	70.2	6.9	12.8	8.4
34.1 ± 3.9	Italy	Parmegiani/2009 (21)	Retrospective	SF	424	2,608	510	70.7	82.6	8.2	16.3	8.8
34.5 ± 3.8	Korea	Yoon/2003 (22)	Prospective	VF	34	474	34	68.6	71.7	5.6	22.2	22.2
33.6 ± 4.3	Colombia	Lucena/2006 (23)[b]	Retrospective	VF	37	179	37	81.0	85.8	3.5	10.8	10.8
32.5 ± 5.8	Korea	Yoon/2007 (24)	Prospective	VF	28	364	30	83.0	77.1	14.2	43.3	36.7
35.7 ± 4.9	Italy	Fadini/2009 (25)	Retrospective	VF	46	285	59	78.9	72.8	9.3	19.2	11.5
31.9 ± 4.5	Italy	Ubaldi[c]/2010 (26)	Prospective	VF	105	487	115	89.7	85.4	16.2	31.5	26.1
		Total			1805	13,079	2,265					

Note: For age, mean ± SD. The success rates of IPD available studies were recomputed from the data obtained. SR = survival rate; FR = fertilization rate; IR = implantation rate (sacs/embryos transferred); CP/T = clinical pregnancy/transfer; LB/T = live birth/transfer.

[a] Follow-up data of reference 18, written in a different row because different cryopreservation method is used.

[b] Includes follow-up data.

[c] Includes RCT data from a previous study by the same group (27).

Cil. Live birth probability with egg freezing. Fertil Steril 2013.

Non donor 1805 patients, 2265 cycles, 10 studies

Cil &Oktay F&S 2013

Live birth rate declines by age for oocyte cryopreservation cycles regardless of the cryopreservation technique

Representative probabilities (%) of live birth for ages 25–42 years, according to number of oocytes thawed, injected, or embryos transferred.

	SF									VF								
	Oocytes thawed			Oocytes injected			Embryos transferred			Oocytes thawed			Oocytes injected			Embryos transferred		
Age (y)	2	4	6	2	4	6	1	2	3	2	4	6	2	4	6	1	2	3
25	12.6	13.5	14.4	12.4	16.0	20.5	7.5	12.4	20.0	28.1	29.7	31.3	24.8	30.9	37.7	13.0	20.7	31.5
26	11.8	12.7	13.5	11.6	15.1	19.4	7.0	11.8	19.0	26.7	28.2	29.8	23.5	29.4	36.0	12.2	19.7	30.1
27	11.1	11.9	12.7	10.9	14.3	18.4	6.6	11.1	18.0	25.3	26.8	28.3	22.3	28.0	34.5	11.6	18.7	28.8
28	10.4	11.2	11.9	10.3	13.4	17.3	6.2	10.5	17.1	24.0	25.4	26.8	21.1	26.6	32.9	10.9	17.7	27.4
29	9.8	10.5	11.2	9.6	12.6	16.4	5.9	9.9	16.2	22.7	24.0	25.4	20.0	25.2	31.4	10.3	16.8	26.2
30	9.1	9.8	10.5	9.1	11.9	15.4	5.5	9.3	15.3	21.4	22.7	24.1	18.9	24.0	29.9	9.7	15.9	24.9
31	8.6	9.2	9.8	8.5	11.2	14.6	5.2	8.8	14.5	20.2	21.5	22.8	17.8	22.7	28.5	9.2	15.0	23.7
32	8.0	8.6	9.2	8.0	10.5	13.7	4.9	8.3	13.7	19.1	20.3	21.6	16.8	21.5	27.1	8.6	14.2	22.6
33	7.5	8.1	8.6	7.5	9.9	12.9	4.6	7.8	12.9	18.0	19.2	20.4	15.9	20.4	25.7	8.1	13.5	21.5
34	7.0	7.5	8.1	7.0	9.3	12.1	4.3	7.3	12.2	17.0	18.1	19.2	15.0	19.3	24.4	7.7	12.7	20.4
35	6.6	7.0	7.6	6.6	8.7	11.4	4.0	6.9	11.5	16.0	17.0	18.1	14.1	18.2	23.1	7.2	12.0	19.3
36	6.1	6.6	7.1	6.2	8.2	10.7	3.8	6.5	10.9	15.0	16.0	17.1	13.3	17.2	21.9	6.8	11.3	18.3
37	5.7	6.2	6.6	5.8	7.7	10.1	3.6	6.1	10.3	14.1	15.1	16.1	12.5	16.2	20.8	6.4	10.7	17.4
38	5.4	5.8	6.2	5.4	7.2	9.5	3.4	5.7	9.7	13.3	14.2	15.1	11.8	15.3	19.6	6.0	10.1	16.5
39	5.0	5.4	5.8	5.1	6.7	8.9	3.1	5.4	9.1	12.5	13.3	14.2	11.1	14.4	18.6	5.6	9.5	15.6
40	4.7	5.0	5.4	4.7	6.3	8.3	3.0	5.1	8.6	11.7	12.5	13.4	10.4	13.6	17.5	5.3	9.0	14.8
41	4.4	4.7	5.0	4.4	5.9	7.8	2.8	4.8	8.1	11.0	11.8	12.6	9.8	12.8	16.6	5.0	8.5	14.0
42	4.1	4.4	4.7	4.1	5.5	7.3	2.6	4.5	7.6	10.3	11.0	11.8	9.2	12.0	15.6	4.7	8.0	13.2

Cil. Live birth probability with egg freezing. Fertil Steril 2013.

Estimated probibilities of live birth for vit. oocytes were higher than slowly frozen

Cil &Oktay F&S 2013

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) [a]
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) [b]	1.7 (1.6–1.8) [b]
Live-birth rate/embryo transfer		
n/total	73/220	108/318
% (95% CI)	33.2 (25.7–38.0)	34.0 (28.7–39.1)
Live-birth rate/patient		
n/total	73/242	108/482
% (95% CI)	30.2 (24.3–35.9)	22.4 (18.7–26.1)
Cumulative live-birth rate/patient[c]		
n/total	88/242	114/482
% (95% CI)	36.4 (30.3–42.4) [d]	23.7 (19.9–27.4) [d]

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

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

[c]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? Systematic review of outcome data

First author, year of publication, country	Study period	Freezing protocol	Live births	Duration of gestation (weeks)	Weight (g)	Comments
Kuleshova,, 1999, Italy	1998	Vitrification	1	37	3,500	Normal female karyotype
Katayama, 2003, Japan	2002	Cryotop vitrification	1	NA	6-pound, 9-ounce	Healthy
Yoon., 2003, Korea	1997-2002	Vitrification	5 singletons 1 set of twins	NA	NA	Healthy (4 had amniocentesis, all normal)
Kuwayama, 2005, Japan	NA	Cryotop vitrification	7	NA	NA	Healthy
Kyono, 2005 Japan	NA	Vitrification	1	NA	3,000	Healthy
Antinori, 2007, Italy	2004-2006	Cryotop vitrification	3	NA	NA	Healthy
Chen, 2008, China	NA	Cryoloop Vitrification	1	38	3,090	Normal karyotype
Chian, 2008, Canada	NA	Cryoleaf or cryptop vitrification	151 singletons 49 multiples	Singletons 37+3 Multiples 35+5	Singletons 2,920±370 Multiples 2,231±550	Congenital anomalies 2.5 % (1 biliary atresia, 1 clubfoot, 1 skin hemangioma, 2 ventricular septal defects)
Total number of infants with some information of health status			221			

Wennerholm et al.,HR 2009

Safety of Oocyte Vitrification???

Table 1. Obstetric and perinatal outcomes and incidence of congenital malformations.

Characteristic	*All pregnancies (n = 165)*	*Singleton pregnancies (n = 137)*	*Multiple gestation pregnancies (n = 28)*
Mean gestational age (weeks + days)	37 + 1	37 + 3	35 + 5
No. of deliveries at 34–37 weeks (%)	46 (30)	30 (22)	16 (57)
No. of deliveries at <34 weeks (%)	10 (6)	6 (4)	4 (14)
	All newborns (n = 200)	*Singleton newborns (n = 141)*	*Multiple gestation newborns (n = 59)*
Birth weight (mean ± SEM) (g)	2784 ± 37	2920 ± 37	2231 ± 55
No. of LBW (%)	68 (34)	24 (17)	44 (74)
No. of VLBW (%)	4 (2)	1 (0.7)	3 (5)
Median Apgar score at 1 min	8	9	8
Median Apgar score at 5 min	10	10	10
Incidence of congenital anomalies			
Biliary atresia	1	0	1
Club foot	1	1	0
Skin hemangioma	1	1	0
Ventricular septal defect	2	0	2
Total (%)	5 (2.5)	2 (1.4)	3 (5.1)

LBW = low birth weight, 1500–2500 g; VLBW = very low birth weight, <1500 g.
SEM = standard error of mean.

Chian et al., RBM Online 2008,

	OS	
Group characteristics	**Singleton pregnancies (n = 9)**	**Multiple gestation pregnancies (n = 6)**
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)	3193.7 ± 376.8	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 vitrifed oocytes by microarray-based CCS on trophoectoderm biopsy, Forman et al., F&S 2012

Conclusions

- **Oocyte vitrification currently offers advantageous and safe clinical results in diverse populations, such as oocyte receipients and typical infertile patients, thus making it very effective fertility preservation option for medical and non-medical reasons**

- **It could be considered as a second breakthrough and watershed in ART after ICSI constituting a giant step toward its definitive validation as a strategy for fertility treatment**

Concerns regarding Vitrification

- **Majority of the articles published on the clinical efficieny of vitrification for human cells utilized open carriers. And so LN2 still remains to be a potential source of contamination since the technique is based on direct contact between the vitrification solution containing cryoprotectant agents and LN2. So from a clinical point of view:**

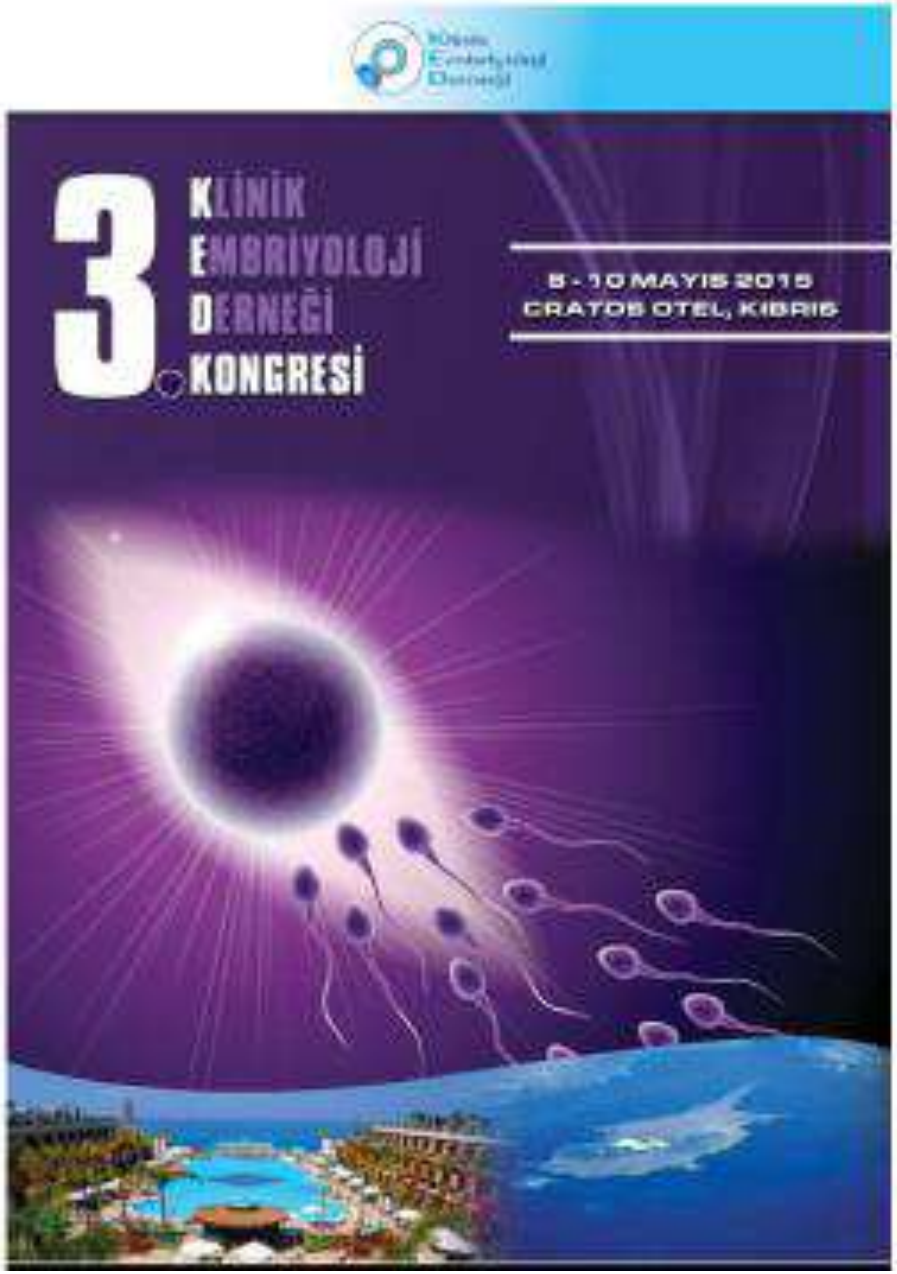
*** Closed systems to avoid contamination are suggested, especially based on the new regulations of EUTCD. Few randomized clinical trials had shown similar efficiency..(Kuwayama RBM 2005-embryos, Van Landuyt HR 2011-blasts.,Stoop RBM 2012-oocytes)

*** Storage of cells in the vapour phase of N2 instead of LN2(Cobo F&S 2010)

*** Sterilization of LN2 (Parmegiani RBM, HR 2011)

- **Safety of vitrification solutions with high concentrations of cryoprotectants??Low toxicity vitrification solutions must be designed in the future**

- **Genetical structure of the vitrified cell?? Chromosal abnormalities, gene expressions ..... More comparitive studies with fresh cells are needed to prove the safety of the technique**



KONUŞMACILAR

- Jacques Cohen, Amerika
- David Gardner Avusturalya
- Thorir Hardarson, İsveç
- Juergen Liebermann Amerika
- Denny Sakkas Amerika
- Carlos Simon İspanya

- Barış Ata Türkiye

KONULAR

- IVF lab. yeni teknolojiler
- IVF evrimi
- IVF başarısında klinisyen/emb. rolü
- IVF Kültür solüsyonları evrimi
- Embriyo seçimi; Klasik/Morfometrik/genetik yöntem?
- IVF'de Kriyoprezervasyonun evrimi