Vitrification embryonnaire et ovocytaire
Etat des lieux, évolutions ?

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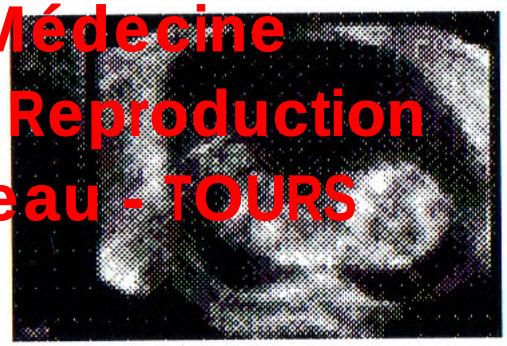

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Article 31

L'article L. 2141-1 du même code est ainsi modifié :

1° Le premier alinéa est remplacé par cinq alinéas ainsi rédigés :

« L'assistance médicale à la procréation s'entend des pratiques cliniques et biologiques permettant la conception *in vitro,* la conservation des gamètes, des tissus germinaux et des embryons, le transfert d'embryons et l'insémination artificielle. La liste des procédés biologiques utilisés en assistance médicale à la procréation est fixée par arrêté du ministre chargé de la santé après avis de l'Agence de la biomédecine. Un décret en Conseil d'Etat précise les modalités et les critères d'inscription des procédés sur cette liste. Les critères portent notamment sur le respect des principes fondamentaux de la bioéthique prévus en particulier aux articles 16 à 16-8 du code civil, l'efficacité, la reproductibilité du procédé ainsi que la sécurité de son utilisation pour la femme et l'enfant à naître. L'Agence de la biomédecine remet au ministre chargé de la santé, dans les trois mois après la promulgation de la loi n° 2011-814 du 7 juillet 2011 relative à la bioéthique, un rapport précisant la liste des procédés biologiques utilisés en assistance médicale à la procréation ainsi que les modalités et les critères d'inscription des procédés sur cette liste.

« Toute technique visant à améliorer l'efficacité, la reproductibilité et la sécurité des procédés figurant sur la liste mentionnée au premier alinéa du présent article fait l'objet, avant sa mise en œuvre, d'une autorisation délivrée par le directeur général de l'Agence de la biomédecine après avis motivé de son conseil d'orientation.

« Lorsque le conseil d'orientation considère que la modification proposée est susceptible de constituer un nouveau procédé, sa mise en œuvre est subordonnée à son inscription sur la liste mentionnée au même premier alinéa.

« La technique de congélation ultra-rapide des ovocytes est autorisée.

« VI. – A titre exceptionnel, des études sur les embryons visant notamment à développer les soins au bénéfice de l'embryon et à améliorer les techniques d'assistance médicale à la procréation ne portant pas atteinte à l'embryon peuvent être conduites avant et après leur transfert à des fins de gestation si le couple y consent, dans les conditions fixées au IV. »

La vitrification embryonnaire peut entrer dans le cadre d'amélioration d'un procédé existant : la congélation embryonnaire

- **VITRIFICATION = Congélation ultra rapide**

=> Constitution d'un état vitreux, SANS cristaux intrac.

- **Etat amorphe obtenu en combinant :**
  - **Cryoprotecteurs à concentrations élevées (X6)**
  - **Vitesse de refroidissement ultra-rapide**

Système OUVERT : ≈ - 20 000 °C / minute
Système FERME : - 1000 à 2 000 °C / minute

Système OUVERT : ≈ - 20 000 °C / minute
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	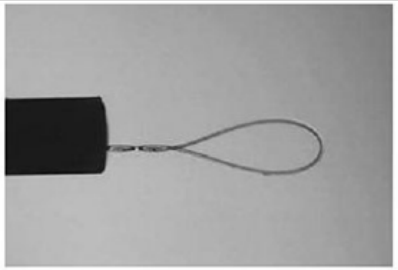	
	Cryoloop	Rapid- i
Description	Nylon loop attached to vial cap	Plastic stick with capillary sized hole
Type of carrier	Open-Direct contact between embryos and LN	Closed-No contact between embryos and LN

- **Système ouvert :**
  - **Risque (?) de contamination par contact direct avec N2 liquide ou d'autres échantillons**

Bielanski 2000, 2009

- **Aperçu de la situation en France**
- **Vitrification embryonnaire précoce**
- **Vitrification embryonnaire tardive**
- **Vitrification ovocytaire**
- **Applications, évolutions**

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Enquête BLEFCO

Dix-huitièmes Journées nationales de la Fédération française d'étude de la reproduction
(Rouen, 25–27 septembre 2013)

Vitrification embryonnaire : état des pratiques en France par les BLEFCO☆

Embryo vitrification: French clinical practice analysis for BLEFCO

L. Hesters [a,*], N. Achour-Frydman [a], J. Mandelbaum [b], R. Levy [c]

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- **Nombre de centres participants :**
  - * Enquête 11/2011 : 36 centres/106 => 16 centres vitrifiant
  - * Enquête 12/2012 : 54 centres/106 => 44 centres vitrifiant

- **Vitrification mise en place pour :**
  - Blastocystes 40/44 (91%)
  - Ovocytes 31/44 (70%)
  - Embryons précoces 25/44 (57%)
  - Zygotes 11/44 (25%)

- **Type de milieux utilisés :**
  - Irvine ® 32/44 (73%)
  - Vitrolife ® 9/44
  - Origio ® / Fertipro ® / Kitazato ® 3/44

- **Marque des paillettes utilisées :**
  - CBS ® 31/44 (70%)
  - Rapid i ® 11/44
  - Vitrosafe ® / Cryotop ® 2/44

- **Système utilisé :**
  - Fermé 43/44 (98%)
  - Ouvert 1/44

Evolution des pratiques

- **Un tiers des centres avait abandonné la congélation lente**
- **Zygotes**
  - Vitrification 15/25
  - Congélation lente 10/25
- **Embryons précoces**
  - Congélation lente 25/44
  - Vitrification 12/44
  - Congélation et vitrification 7/44
- **Blastocystes**
  - Vitrification 34/44
  - Congélation lente 7/44
  - Congélation et vitrification 3/44

- **Aperçu de la situation en France**
- **Vitrification embryonnaire précoce**
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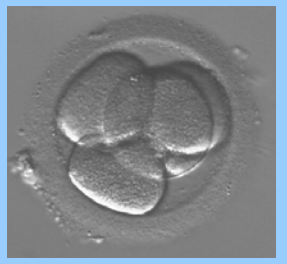
Enquête BLEFCO 2012



- **Les résultats**

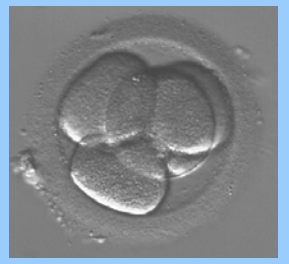
	Congélation lente	Vitrification
Nb embryons décongelés	12532	1565
Tx survie	70%	88%
Tx Grossesse / transfert	18.8%	24.7%

Embryons précoces : Congél. lente vs. vitrification

	Cryoprotectants	Survival rate (%)		Cryopreservation IR (%)		Fresh IR (%)
		≥50% intact	Fully intact	≥50% intact	Fully intact	
Slow cooling						
Edgar *et al.* (2000), D2	1.5 M PROH, 0.1 M sucrose	78.3	55.5	10.3	11.3	11.4
Veeck (2003), D2	1.5 M PROH, 0.1 M sucrose	78.6		15.2		
Gabrielsen *et al.* (2006), D2	1.5 M PROH, 0.1 M sucrose	76.5		10.4		
Mandelbaum *et al.* (1998), D2	1.5 M PROH, 0.1 M sucrose	73		16[a]		
Comparison of two slow cooling methods						
Edgar *et al.* (2009), D2	1.5 M PROH, 0.1 M sucrose	78.5	54.6	17.5		
	1.5 M PROH, 0.2 M sucrose	92.6	80.5	22.1		
Wood *et al.* (2011), D2	1.5 M PROH, 0.1 M sucrose	80	57	NR		
	1.5 M PROH, 0.3 M sucrose	90	73	NR		
Optimal vitrification						
Rama Raju *et al.* (2009), D3	40% EG, 0.6 M sucrose	90.4		18.1		23.5
Desai *et al.* (2007), D3	15% EG, 15% DMSO, 0.65 M sucrose, 10 mg/ml Ficoll	85		20		
Comparison of slow cooled and vitrified						
Kuwayama *et al.* (2005b), D2 slow	1.5 M PROH, 0.1 M sucrose	91		32[a]		
Kuwayama *et al.* (2005b), vit	15% EG, 15% DMSO, 0.5 M sucrose	98		27[a]		
Wilding *et al.* (2010), D3	Cleavage Cryopreservation Kit (Cook)	87		13.5		
	Blastocyst Vitrification Kit (Cook)	93		14.3		
Balaban *et al.* (2008), D3 slow	Freeze- Kit 1 (Vitrolife)	88.7	45.7	NR		
Balaban *et al.* (2008), Vit	16% EG, 16% PROH, 0.65 M sucrose, 10 mg/ml Ficoll	94.8	73.9	29.7		

Edgar and Gook, HR Update 2012

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



Alpha Scientists in Reproductive Medicine [1,*]

[1] Meeting participants: Başak Balaban* (Assisted Reproduction Unit, American Hospital, Istanbul, Turkey), Veronica Bianchi (Tecnobios Procreazione, Bologna, Italy), Virginia Bolton (Assisted Conception Unit, Guy's Hospital, London, UK), Ana Cobo (Instituto Valenciano de Infertilidad, Valencia, Spain), Thomas Ebner (Kinderwunsch Zentrum Linz, Austria), David Edgar (Reproductive Services/Melbourne IVF, Royal Women's Hospital and Department of Obstetrics & Gynaecology, University of Melbourne, Victoria, Australia), Martin Greuner (Zentrum für gynäkologische Endokrinologie und Reproduktionsmedizin (IVF-SAAR), Saarbrücken, Germany), Stanley Leibo (Department of Biological Sciences, University of New Orleans, New Orleans, LA, USA), David Mortimer (Oozoa Biomedical Inc, West Vancouver BC, Canada), Zsolt Peter Nagy (Reproductive Biology Associates, Atlanta, GA, USA), Lodovico Parmegiani (Reproductive Medicine Unit-GynePro Medical Centers, Bologna, Italy), Tammie Roy (Genea, Kent Street, Sydney NSW, Australia), Alan Thornhill (The London Bridge Fertility, Gynaecology and Genetics Centre, London Bridge, UK), Lisbet Van Landuyt (Centre for Reproductive Medicine, University Hospital, Vrije Universiteit Brussel, Brussels, Belgium), Pierre Vanderzwalmen (IVF Centers Prof Zech – Bregenz, Austria and CHIREC, Braine-l'alleud, Belgium), Matthew (Tex) VerMilyea (Shady Grove Fertility Reproductive Science Center, Rockville, MD, USA), Maureen Wood (University of Aberdeen, Aberdeen, Scotland, UK)

* Corresponding author. *E-mail address:*alpha2@bluewin.ch (B Balaban)

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.

Alpha group, RBM Online 2012

Embryons précoces : Devenir des enfants

Table II Obstetric and neonatal outcomes in singleton pregnancies after transfer of vitrified, slow freeze and fresh Day 3 embryos.

	Vitrified (n = 545)	Slow freeze (n = 986)	Fresh (n = 1914)	Test between groups, P-value	
				Vitrified versus slow freeze	Vitrified versus fresh
Maternal age, (years)	32.4 ± 3.5 (22–45)	32.1 ± 3.1 (22–44)	31.9 ± 3.3 (21–43)	0.537	0.429
BMI (kg/m^2)	21.4 (18.3–24.0)	21.6 (17.4–24.3)	21.6 (18.1–24.1)	0.775	0.801
Primiparous	295 (54.1%)	523 (53.0%)	1072 (56.0%)	0.683	0.436
Educational level, university	218 (40.0%)	366 (37.1%)	861 (45.0%)	0.267	0.039
Babies born	545	986	1914		
Live borns	543	982	1907		
Gender					
Male	276 (50.6%)	491 (49.8%)	959 (50.1%)	0.752	0.825
Female	269 (49.4%)	495 (50.2%)	955 (49.9%)		
Spontaneous vaginal delivery	60 (11.0%)	107 (10.9%)	189 (9.9%)	0.977	0.837
Vacuum or forceps extraction	2 (0.4%)	3 (0.3%)	7 (0.4%)		
Caesarean delivery	483 (88.6%)	876 (88.8%)	1718 (89.8%)		
Hypertensive disorder	27 (5.0%)	41 (4.2%)	74 (3.9%)	0.469	0.259
Hypertension	6 (1.1%)	8 (0.8%)	13 (0.7%)	0.569	0.321
Pre-eclampsia	21 (3.9%)	33 (3.3%)	61 (3.2%)	0.607	0.445
Gestational diabetes	18 (3.3%)	29 (2.9%)	55 (2.9%)	0.695	0.602
Placenta previa	2 (0.4%)	5 (0.5%)	6 (0.3%)	1.0	1.0
Abruptio placenta	1 (0.2%)	3 (0.3%)	5 (0.3%)	1.0	1.0
Gestational age (weeks)	38.7 ± 1.7 (25.6–42.5)	38.7 ± 1.7 (24.3–42.6)	38.7 ± 1.7 (25–42.8)	>0.05	>0.05
32–37	41 (7.5%)	91 (9.2%)	149 (7.8%)	0.255	0.840
28–32	4 (0.7%)	8 (0.8%)	6 (0.3%)	1.0	0.243
<28	1 (0.2%)	1 (0.1%)	4 (0.2%)	1.0	1.0
Birthweight (g)	3455.3 ± 482.0 (790–5450)	3352.3 ± 500.7 (810–5100)	3355.8 ± 490.9 (750–5400)	0.0001	0.0001
1500–2500	10 (1.8%)	34 (3.5%)	49 (2.6%)	0.070	0.329
<1500	2 (0.4%)	6 (0.6%)	8 (0.4%)	0.719	1.0
SGA	8 (1.5%)	20 (2.0%)	35 (1.8%)	0.433	0.571
Apgar score < 7 at 5 min	15 (2.8%)	45 (4.6%)	56 (2.9%)	0.088	0.831
Transferred to NICU	44 (8.1%)	71 (7.2%)	153 (8.0%)	0.535	0.952
Perinatal mortality	2 (0.4%)	4 (0.4%)	7 (0.4%)	1.0	1.0
Stillborns	0	1	1		
Early neonatal mortality	2	3	6		

SGA: small for gestational age; NICU: neonatal intensive care unit.

Liu et al, HR 2012

- Aperçu de la situation en France
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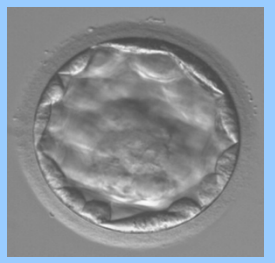
Enquête BLEFCO 2012



- **Les résultats**

	Congélation lente	Vitrification
Nb blastocystes décongelés	4218	2698
Tx survie	71%*	88%*
Tx Grossesse / transfert	18.5%* [6.3-33.3]	29.5%* [0-50%]

* p<0.001

Blastocystes : Congélation lente vs. vitrification

Tx SURVIE	Congélation lente	Vitrification	Collapse	*p*
Huang 2005	57% (72)	84%(81)	NON	*<0.05*
Kuwayama 2005	84% (156)	90% (6328)	NON	*<0.05*
Stehlik 2005	83% (71)	100% (41)	NON	*<0.05*
Lieberman-Tucker 2006	92% (570)	97% (547)	NON	*NS*
Kuc 2010	83% (189)	84% (58)	NON	*NS*
CHRU Tours 2013	79% (275)	89% (220)	NON	*<0.05*

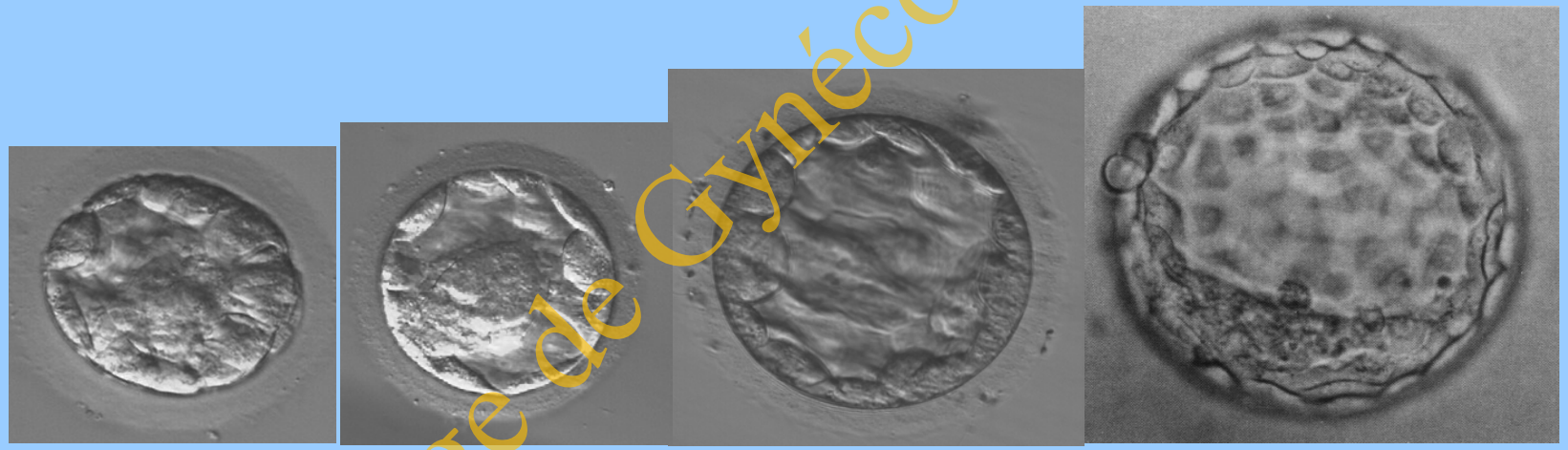
% Gros. clin/ transfert	SBT	Congélation lente	Vitrification	*p*
Kuwayama 2005	?	51% (98)	53% (4745)	*NS*
Stehlik 2005	NON	17% (24)	50% (20)	*<0.05*
Lieberman et Tucker 2006	NON	43% (254)	46% (254)	*NS*
Kuc 2010	NON	26%(83)	50% (58)	*<0.05*
CHRU Tours 2013	NON	24% (180)	34% (155)	*NS*

Blastocystes : Influence du jour de vitrification

Taux de Survie	Day 5	Day 6	*p*
Mukaida 2003	87% (569)	55% (156)	*<0.05*
Hiraoka 2009	100% (214)	98% (145)	*NS*
Lieberman 2009	96% (1373)	96% (1357)	*NS*
Van Landuyt 2011	81% (864)	70% (321)	*<0.05*
Cobo 2012	96% (1033)	98% (929)	*NS*
CHRU Tours 2013	93% (137)	82% (67)	*<0.05*

% Gros. clin/ transf	Day 5	Day 6	*p*
Hiraoka 2009	54% (144)	53% (100)	*NS*
Lieberman 2009	58% (678)	42% (720)	*<0.05*
Van Landuyt 2011	17% (406)	16% (124)	*NS*
Cobo 2012	46% (649)	43% (589)	*NS*
Hashimoto 2013	57% (608)	47% (270)	*<0.05*
Kang 2013	42% (737)	30% (154)	*<0.05*
CHRU Tours 2013	40% (94)	19% (43)	*<0.05*

Influence du stade lors de la vitrification



B1 B2 B3 B4-B5

TAUX de SURVIE	Early Blastocyst	Expanded Blastocyst	Hatched Blastocyst
Cho 2002	89% (54)	77% (60)	
Vanderzwalmen 2002 (Day 5)	80% (41)*	29%(71)*	
Mukaida 2003 (Day 5)	92% (119)*	87% (361)*	82% (89)*
Ebner 2009 (Day 5)	84% (86)*	74% (53)	68% (134)*
Cobo 2012 (Day 5)	94% (458)	98% (549)*	89% (72)*
CHRU Tours 2013 (Day 5)	97% (17)	87% (65)	81% (27)

Tx Gros. Clin. / transfert	Early Blastocyst	Expanded Blastocyst	Hatched Blastocyst
Vanderzwalmen 2002 (Day 5)	25% (16)	14% (7)	
Mukaida 2003 (Day 5)	45% (38)	37% (107)	35% (20)
Ebner 2009 (Day 5)	45% (42)	30% (23)	48% (48)
Cobo 2012 (Day 5)	32% (285)	44% (349)	44.4% (41)
CHRU Tours 2013 (Day 5)	6% (17)	38% (65)	8% (27)

* = $p<0.05$

Blastocystes expansés :

- Risque accru de formation de glace
- Perméabilité diminuée aux cryoprotecteurs

⇒ Idée d'induire un collapse artificiel

Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human blastocysts

T.Mukaida[1,3], C.Oka[2], T.Goto[2] and K.Takahashi[1]

Blastocoele collapse by micropipetting prior to vitrification gives excellent survival and pregnancy outcomes for human day 5 and 6 expanded blastocysts

Kenichiro Hiraoka[1], Kaori Hiraoka, Masayuki Kinutani and Kazuo Kinutani

Kinutani Women's Clinic, 2-1-4-3F, Ohtemachi, Naka-ku, Hiroshima 730-0051, Japan

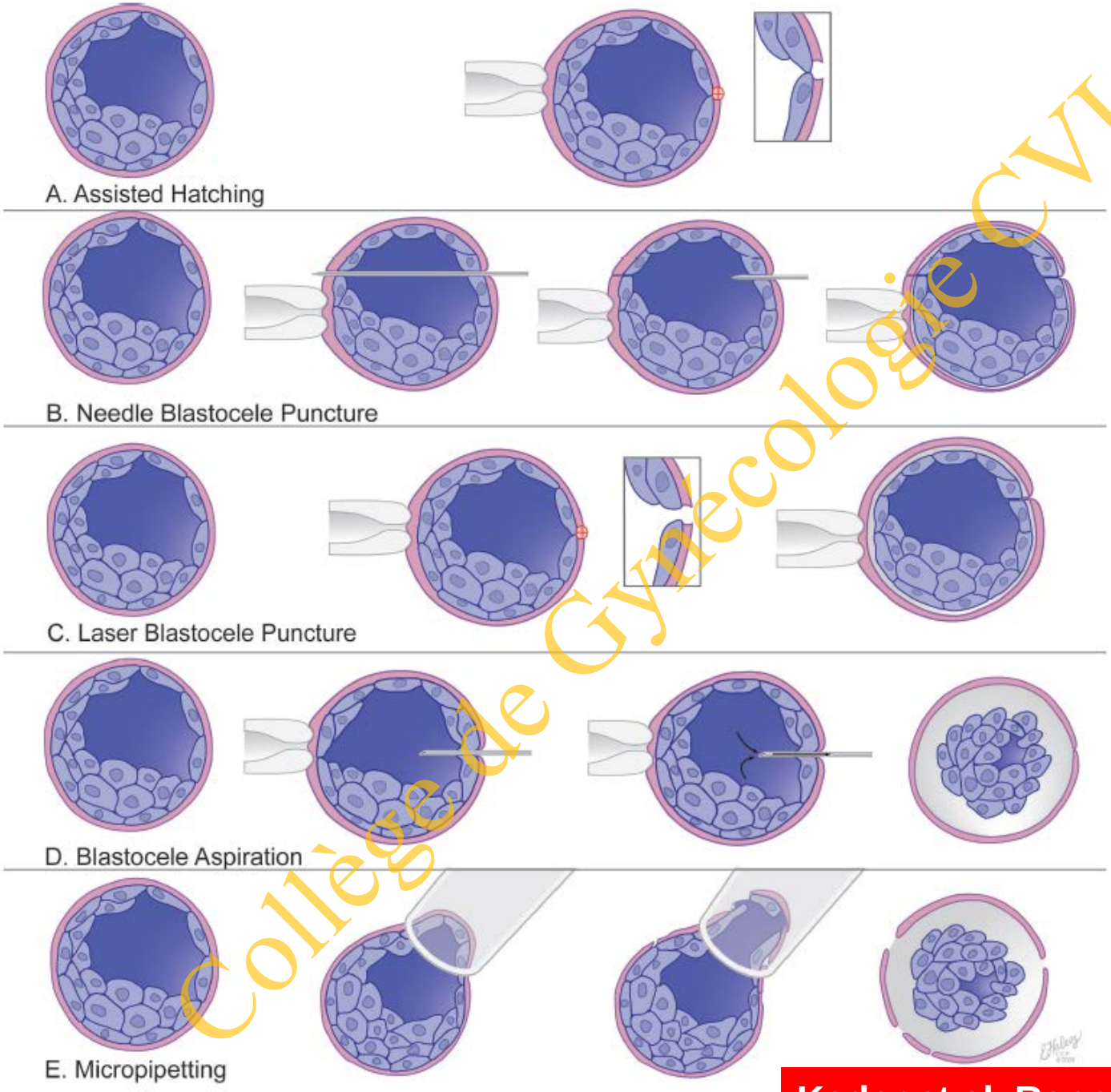


Figure 1

Kader et al, Reprod Biol End 2009

Mukaida 2006	Expanded / Hatched Blastocysts	Expanded / Hatched Blastocysts + collapse	*p*
Tx Survie	85% (339)	97% (502)	*<0.05*
Tx grossesse / transfert	34% (85)	60% (266)	*<0.05*

Iwayama 2011	Expanded Blastocysts	Expanded Blastocysts + collapse	*p*
Tx Survie	97% (?)	100% (?)	*NS*
Tx grossesse /transfert	34% (85)	60% (129)	*<0.05*

Raju 2009	Early Blastocyst	Expanded blastocyst + collapse	*p*
Tx Survie	95% (576)	81% (370)	*<0.05*
Tx grossesse / transfert	38% (281)	32% (193)	*NS*

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

Alpha Scientists in Reproductive Medicine [1,*]

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* Corresponding author. *E-mail address:*alpha2@bluewin.ch (B Balaban)

Blastocyste survivant si ≥ 75% des cellules perçues comme intactes

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.

Alpha group, RBM Online 2012

Blastocystes : Devenir des enfants

	Wikland 2010		
Grossesses uniques	**Blastocyste frais**	**Blastocyste vitrifié**	***p***
n	**199**	**103**	
Age gestationnel (AG)	**40.1**	**40.3**	***NS***
AG < 37 semaines	**7%**	**7%**	***NS***
Poids naissance (g)	**3510**	**3650**	***<0.05***
Poids < 2500 g	**5%**	**7%**	***NS***
Tx de malformations	**2%**	**1%**	***NS***
Mortalité périnatale	**0.5%**	**1%**	***NS***

Blastocystes : Devenir des enfants

	Feng 2012		
Grossesses uniques	Blastocyste frais	Blastocyste vitrifié	*p*
n	252	142	
Age gestationnel (AG)	38.2 ± 4.6	38.6 ± 1.6	*NS*
AG < 37 semaines	10%	10%	*NS*
Poids naissance (g)	3155 ± 590	3103 ± 515	*NS*
Poids < 2500 g	5.6%	7.9%	*NS*

human reproduction

ORIGINAL ARTICLE *Embryology*

The time aspect in storing vitrified blastocysts: its impact on survival rate, implantation potential and babies born

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2013

Absence de formation de structures cristallines => Stabilité dans le temps des blastocystes vitrifiés ?

Analyse rétrospective de 603 transferts (2009-2012)
Système fermé

Table II SRs of blastocysts and clinical outcome following transfer of vitrified blastocysts after different storage times.

	Storage groups							P-value
Mois	0-3	3-6	6-12	12-24	24-36	36-48	48-72	
SR	283/341 (83.0%)	488/543 (89.9%)	274/325 (84.3%)	250/305 (82.0%)	243/297 (81.8%)	86/98 (87.8%)	69/83 (83.1%)	n.s.
embryos transferred (*n*)	186 (1.9/ET)	316 (1.9/ET)	186 (1.9/ET)	153 (1.7/ET)	149 (1.7/ET)	47 (1.7/ET)	40 (1.5/ET)	$P < 0.001$
IR (*n*; %)	46 (24.7)	65 (20.6)	36 (19.4)	39 (25.5)	47 (31.5)	15 (31.9)	12 (30.0)	n.s.
PR (*n*; %)	48 (48.0)	65 (38.5)	39 (39.4)	46 (50.0)	48 (53.3)	13 (48.2)	12 (46.2)	n.s.
cPR (*n*; %)	40 (40.0)	51 (30.2)	33 (33.3)	31 (33.7)	43 (47.8)	11 (40.7)	10 (38.5)	n.s.
BR (*n*; %)	33 (33.0)	47 (27.8)	28 (28.3)	30 (32.6)	35 (38.9)	11 (40.7)	7 (26.9)	n.s.

SR, survival rate; IR, implantation rate; PR, pregnancy rate; cPR, clinical PR; BR, birth rate. PR, cPR and BR are calculated 'per cycle'.

Table III Characteristics of the children born.

	Group						
	I	II	III	IV	V	VI	VII
Children born	46	55	30	35	37	12	9
Singletons	20	41	26	26	33	10	5
Multiples	26	14	4	9	4	2	4
Medically induced abortions	–	2[a]	–	–	–	–	1[b]
Stillbirths	2	–	–	–	–	–	–
Single malformation	–	–	–	–	–	1[c]	–
Multiple malformations	–	–	–	1[d]	–	–	–

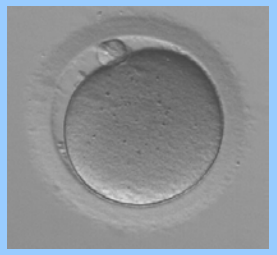
Singletons and multiples born after blastocyst VIT.
[a]Both due to trisomy 21.
[b]After diagnosis of multiple malformations
[c]One twin child born with cleft palatine.
[d]Child born with multiple malformations, died at the age of 4 weeks.

Souris (6 mois)
Porc (3 ans)
Lapin (15 ans)

Wirleitner et al, HR 2013

- Aperçu de la situation en France
- Vitrification embryonnaire précoce
- Vitrification embryonnaire tardive
- Vitrification ovocytaire
- Applications, évolutions

Enquête BLEFCO 2012



- **Les résultats**

	Système fermé	Système ouvert
Nb centres	18	1
Nb ovocytes décongelés	??	??
	660	
Tx survie	69%	76%
	[75-97%]	
Tx fécondation	70%	71%
Tx Grossesse / transfert	16%	27%
	[38-65%]	

Ovocytes : Congélation lente vs. vitrification

	Cryoprotectants	Survival rate (%)	Cryopreservation IR (%)		Fresh IR (%)
			Cleavage stage ET	Blastocyst ET	
Optimal slow cooling					
Gook and Edgar (2011)	1.5 M PROH, 0.2 M sucrose	75.8	30.0 (<38 years)		26.0 (<38 years)
Yang *et al.* (2002), donor	1.5 M PROH, 0.2 M sucrose	70.9	25.3		43.4
Bianchi *et al.* (2007)	1.5 M PROH, 0.2 M sucrose	75.1	16.7		17.3
Konc *et al.* (2008b)	1.5 M PROH, 0.3 M sucrose	76	15.4[a]		18
Ferraretti *et al.* (2010)	1.5 M PROH, 0.3 M sucrose	71.8	18.9 (≤35 years)		
Parmegiani *et al.* (2009)	1.5 M PROH, 0.3 M sucrose	71.6	15.1		
Optimal vitrification					
Antinori *et al.* (2007)	15% EG, 15% DMSO, 0.5 M sucrose	99.4	13.2		10.3
Rienzi *et al.* (2010)	15% EG, 15% DMSO, 0.5 M sucrose	96.7–89.7	27.3 (≤34 years)		30.0 (≤34 years)
Ubaldi *et al.* (2010)					
Almodin *et al.* (2010)	15% EG, 15% DMSO, 0.5 M sucrose	84.9	14.9		21.3
Cobo *et al.* (2010a), donor	15% EG, 15% DMSO, 0.5 M sucrose	92.5	39.9		40.9
Trokoudes *et al.* (2011), donor	15% EG, 15% DMSO, 0.5 M sucrose	91.4	24.7		25.6
Cobo *et al.* (2008a), donor	15% EG, 15% DMSO, 0.5 M sucrose	96.9		40.8	
Nagy *et al.* (2009), donor	15% EG, 15% DMSO, 0.5 M sucrose	89		55.3	47.4
Garcia *et al.* (2011), donor	15% EG, 15% DMSO, 0.5 M sucrose	89.4		43.9	42.9
Comparison of slow cooled and vitrified (vit)					
Smith *et al.* (2010), slow	1.5 M PROH, 0.3 M sucrose	65	13[a,b]		
Smith *et al.* (2010), vit	15% EG, 15% DMSO, 0.5 M sucrose	75	38[a,b]		
Noyes *et al.* (2010), slow	1.5 M PROH, 0.3 M sucrose	85	NR (mixed transfers)		
Noyes *et al.* (2010), vit	15% EG, 15% DMSO, 0.5 M sucrose	88	NR (mixed transfers)		
Fadini *et al.* (2009), slow	1.5 M PROH, 0.3 M sucrose	57.9	4.3		
Fadini *et al.* (2009), vit	EG, PROH, sucrose (Medicult)	78.9	9.3		

Edgar and Gook, HR Update 2012

Ovocytes : Système ouvert vs. Système fermé

Table 3 CryoTip versus CryoTop groups: embryological data.

Characteristic	*CryoTip (closed) vitrification-warming cycles (n = 51)*	*CryoTop (open) vitrification-warming cycles (n = 53)*	P	*OR (95% CI)*
Warmed oocytes (total)	5.1 ± 2.0 (261)	5.1 ± 1.4 (268)	NS	
Survived oocytes	151/261 (57.9)	222/268 (82.8)	0.001	0.3 (0.2–0.4)
Inseminated oocytes (total)	2.8 ± 1.3 (151)	3.0 ± 0.8 (159)	NS	
Fertilization (2PN) rate	87/151 (57.6)	116/159 (73.0)	0.005	0.5 (0.3–0.8)
Abnormal 2PN morphology	14/87 (16.1)	2/116 (1.7)	0.001	10.9 (2.4–100.8)
Oocytes degenerated post ICSI	16/151 (10.6)	10/159 (6.3)	NS	1.8 (0.8–4.2)
Embryos on day 2 (≥2 cells)	69/87 (79.3)	112/116 (96.6)	0.001	0.1 (0.0–0.4)
Good-quality embryos on day 2	30/87 (34.5)	62/112 (55.4)	0.003	0.6 (0.5–0.9)
Cycles with no viable embryos	24/51 (47.1)	1/53 (1.9)	0.001	46.2 (7.7–976.7)
Clinical pregnancy rate/ warming cycle	4/51 (7.8)	14/53 (26.4)	0.01	0.2 (0.1–0.8)
Clinical pregnancy rate/ patient	4/48 (8.3)	14/49 (28.6)	0.01	0.2 (0.1–0.8)
Implantation rate	4/69 (5.8)	15/112 (13.4)	NS	0.4 (0.1–1.3)
Gestational sacs/ inseminated oocyte	4/151 (2.6)	15/159 (9.4)	0.02	0.3 (0.1–0.8)
Live birth rate/warming cycle	3/51 (5.9)	11/53 (20.8)	NS	0.2 (0.0–1.0)

Values are mean ± SD or *n*/total (%) unless indicated otherwise.
CI = confidence interval; NS = not statistically significant; OR = odds ratio; 2PN = two pronuclei.

Paffoni et al, RBM Online 2011

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

Alpha Scientists in Reproductive Medicine [1,*]

[1] Meeting participants: Başak Balaban* (Assisted Reproduction Unit, American Hospital, Istanbul, Turkey), Veronica Bianchi (Tecnobios Procreazione, Bologna, Italy), Virginia Bolton (Assisted Conception Unit, Guy's Hospital, London, UK), Ana Cobo (Instituto Valenciano de Infertilidad, Valencia, Spain), Thomas Ebner (Kinderwunsch Zentrum Linz, Austria), David Edgar (Reproductive Services/Melbourne IVF, Royal Women's Hospital and Department of Obstetrics & Gynaecology, University of Melbourne, Victoria, Australia), Martin Greuner (Zentrum für gynäkologische Endokrinologie und Reproduktionsmedizin (IVF-SAAR), Saarbrücken, Germany), Stanley Leibo (Department of Biological Sciences, University of New Orleans, New Orleans, LA, USA), David Mortimer (Oozoa Biomedical Inc, West Vancouver BC, Canada), Zsolt Peter Nagy (Reproductive Biology Associates, Atlanta, GA, USA), Lodovico Parmegiani (Reproductive Medicine Unit-GynePro Medical Centers, Bologna, Italy), Tammie Roy (Genea, Kent Street, Sydney NSW, Australia), Alan Thornhill (The London Bridge Fertility, Gynaecology and Genetics Centre, London Bridge, UK), Lisbet Van Landuyt (Centre for Reproductive Medicine, University Hospital, Vrije Universiteit Brussel, Brussels, Belgium), Pierre Vanderzwalmen (IVF Centers Prof Zech – Bregenz, Austria and CHIREC, Braine-l'alleud, Belgium), Matthew (Tex) VerMilyea (Shady Grove Fertility Reproductive Science Center, Rockville, MD, USA), Maureen Wood (University of Aberdeen, Aberdeen, Scotland, UK)

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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		

Alpha group, RBM Online 2012

Ovocytes : Devenir des enfants

Supplementary Table II. Slow freezing of oocytes. Neonatal outcome.

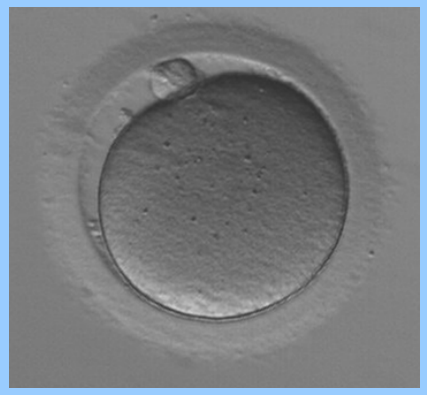
First author, year of publication, country	Study period	Live births	Gestational age (weeks)	Weight (g)	Comments
Porcu., 2000, Italy	1997-2000	16	NA	NA	Healthy
Fosas, 2003, Spain	2001-2003	3 singletons one set of twins	NA	NA	Oocyte donation Healthy
Borini., 2004, Italy	1997-2000	9 singletons two sets of twins	NA	3,19 ± 0.62 (kg)	Healthy; Normal karyotypes; Boys 5, Girls 8; No malformations
Chen, 2005, Taiwan	2001-2004	5	NA	NA	Normal karyotypes
Li, 2005, Taiwan	2003-2005	5 singletons one set of twins	NA	NA	Partly oocyte donation Healthy
La Sala, 2006, Israel	2004-2005	7 singletons	39 (37-42)	3,400 (2,610-4,325)	Boys 3, Girls 4 Healthy
Levi Setti., 2006, Italy	1999-2003	11 singletons one set of twins	37.1	2,807	Boys 4, Girls 9; Normal karyotypes 6 month follow-up normal
Bianchi., 2007, Italy	2004-2005	4	NA	NA	Healthy
Borini., 2007, Italy	2004-2006	52 singletons 4 sets of twins	NA	NA	2/60 children had developmental abnormalities Choanae atresia 1 Rubinstein-Taydi syndrom 1
De Santis., 2007, Italy	2004-2006	3 singletons	NA	NA	Normal karyotypes
Konc., 2007, Hungary		1 singleton one set of twins	NA	NA	Healthy
11 case reports: Azambuja 2005, De Geyter 2007, Gook 2007, Greco 2008, Kan 2004, Levi Setti 2005, Montag 2006, Notrica 2004, Quintans 2002, Tjer 2005, Yang 2007	1997-2007	12	34-40	2,050-3,632	Healthy
Total number of infants with some information of health status		148			

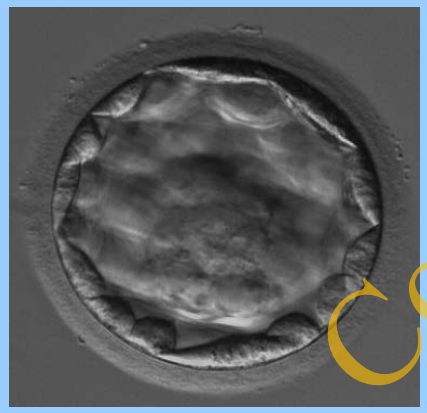
Supplementary Table III. Vitrification of oocytes. Neonatal outcome.

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, 2012 HR

- Aperçu de la situation en France
- Vitrification embryonnaire précoce
- Vitrification embryonnaire tardive
- Vitrification ovocytaire
- Applications, évolutions



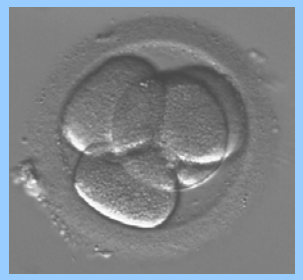


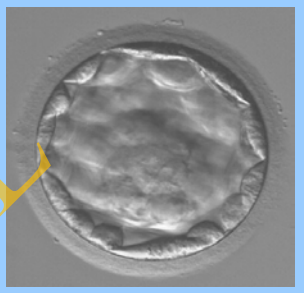
VITRIFICATION

Loi du 7 juillet 2011

-Techniques d'AMP
- Préservation
de la fertilité féminine
-Banques d'ovocytes

- Développement des
transferts mono-embryonnaires
- Désynchronisation
ponction / transfert





- Favoriser le développement des **transferts mono-embryonnaire**
- Favoriser le développement des **transferts différés au stade blastocyste**
- Favoriser la **désynchronisation** entre ponction et transfert embryonnaire

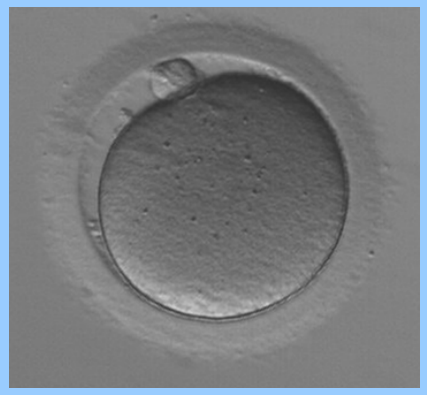
This article has been retracted

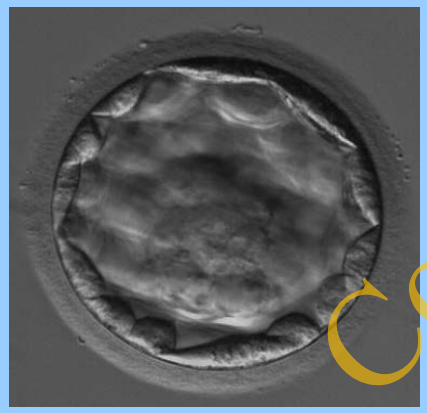
RETRACTED ARTICLE: Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial

Abbas Aflatoonian, Homa Oskouian, Shahnaz Ahmadi, and Leila Oskouian

Author information ▶ Article notes ▶ Copyright and License information ▶

This article has been retracted. See J Assist Reprod Genet. 2013 August 23; 30(9): 1245.





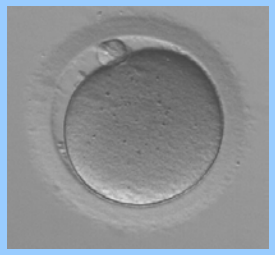
VITRIFICATION

Loi du 7 juillet 2011

-Techniques d'AMP
-Préservation
de la fertilité féminine
-Banques d'ovocytes

- Développement des
transferts mono-embryonnaires
- Désynchronisation
ponction / transfert

Enquête BLEFCO 2012

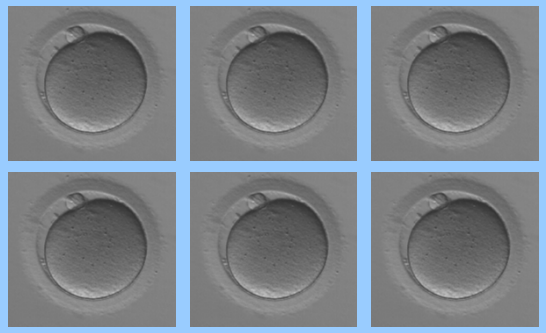


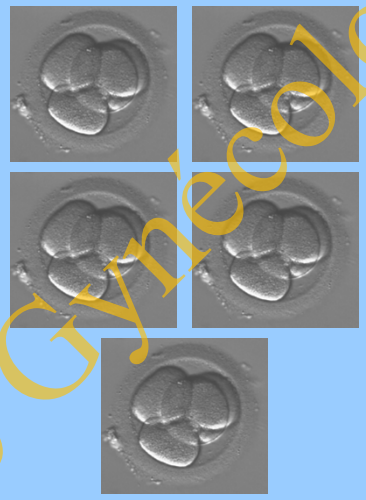
- **La situation :**
  - 31 centres avaient vitrifié
  - 19 centres avaient procédé à des réchauffements

- **Quand est elle proposée ?**
  - Echec de recueil 31/31
  - Préservation fertilité 20/31
  - BT négative 14/31
  - Don ovocyte 6/31
  - Alternative congélation 5/31

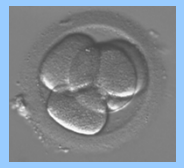
Procédure d'AMP

Mise en fécondation





Transfert



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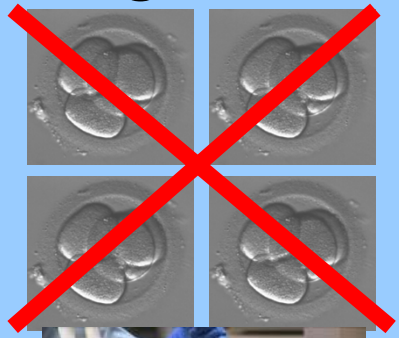
Congélation



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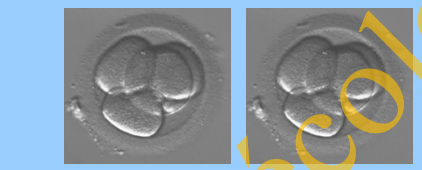
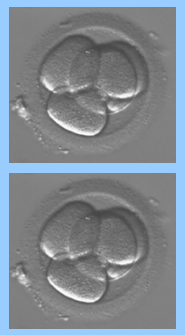
Procédure d'AMP

Mise en fécondation

Transfert





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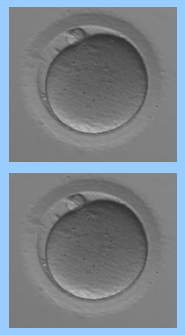
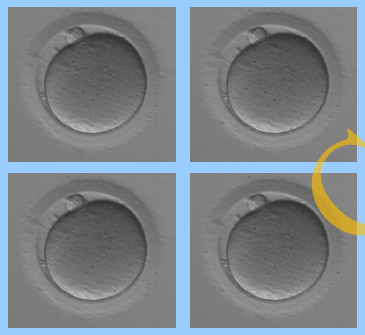
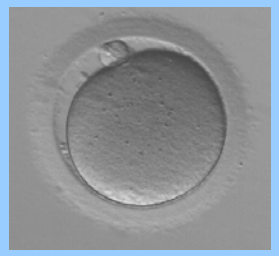


Application en Italie
2004-2009
-Échecs de fécondation
-Pb choix des embryons
-Elévation du Tx de Gros. multiples

Congélation

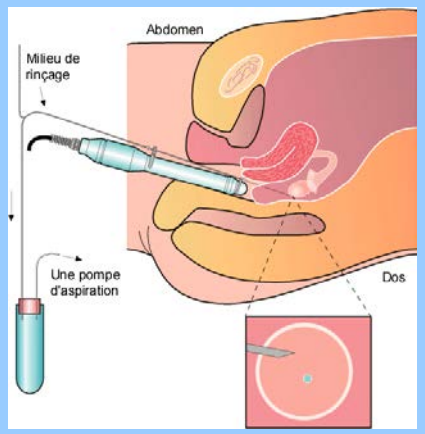


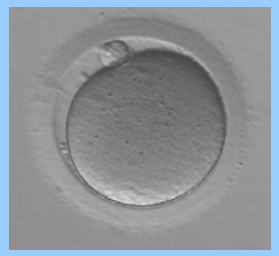
Nécessite une ICSI systématique



Préservation de la fertilité

- **Indication**
  - N°1: Femme jeune, célibataire, laps de temps suffisant pour ttt de stimulation
- **Stimulation ovarienne préalable: 2 à 6 semaines**
  - Souvent incompatible avec urgence thérapeutique
  - Contre-indiquée dans les cancers hormono-dépendants
  - Parfois réponse inadéquate :
    - Plus souvent insuffisante : AEG, type de cancer…





Constitution de banques d'ovocytes

- Rapport IGAS 2011

⇒ Carence du système français dans le don d'ovocytes

- Eviter synchronisation « donneuse-receveuse »
  - Meilleure disponibilité des donneuses
  - Renforcement anonymat / confidentialité

Oocyte cryopreservation for donor egg banking

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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)

Cobo et al, RBM Online 2011

Characteristics of the seven identified commercial egg banks in the United States.

CEB	Freezing technique	Years in existence	No. of donors used to date	No. of currently available oocytes	No. of oocytes recommended
1	Vitrification	8	18	160	6
2	Vitrification	2	100	100	6
3	Vitrification	2	25	900	6
4	Vitrification	1	6	600	7
5	Vitrification	2	70	1,000	6
6	Vitrification	5	15	120	6
7	Slow freeze	7	60	250	4

Quass et al, FS 2013

HUMAN FERTILISATION & EMBRYOLOGY AUTHORITY

Home | A A A | Sitemap | HFEA job vacancies | Contact us | FAQs | Glossary

Search

Patients | Donor-conceived (people & their parents) | Donors (donating for treatment & research) | Clinic staff (& other professionals) | Media (& researchers) | About the HFEA

Home > Media & researchers > Background briefings > About HFEA regulation > Egg sharing schemes

Media & researchers | Press releases and statements | Facts & figures | Background briefings | Analyse our data

Background briefings

- New techniques
- About HFEA regulation
  - Egg sharing schemes
  - Welfare of the child assessment
  - What we do about OHSS
  - Sperm websites
  - Surrogacy
- History of IVF

Egg sharing schemes

What is egg sharing?

- Egg sharing involves a woman having fertility treatment and donating some of her eggs in return for benefits in kind in the form of discounted treatment services, usually a reduced treatment fee.
- There is a need for egg sharing because of the shortage of donated eggs - some women have been known to wait 3-5 years to receive donated eggs. Some egg sharing schemes involve donation for research.
- Clinics are expected to ensure that the donor and the recipient (and, where appropriate, any partners) have full information about the nature of the treatment so they are able to give informed consent. Agreements should be in place between the egg provider and the treatment centre and the egg recipient(s) and the treatment centre.

Back to top

Interested in becoming a donor?

Becoming a donor can be a life-changing decision. To donate, you will need to fulfill certain criteria to establish your suitability.

...more about donating

« *Art. L. 1244-1-1.* – Les médecins gynécologues informent régulièrement leurs patientes sur le don d'ovocytes.

« *Art. L. 1244-1-2.* – Les médecins traitants informent régulièrement leurs patients sur le don de gamètes. »

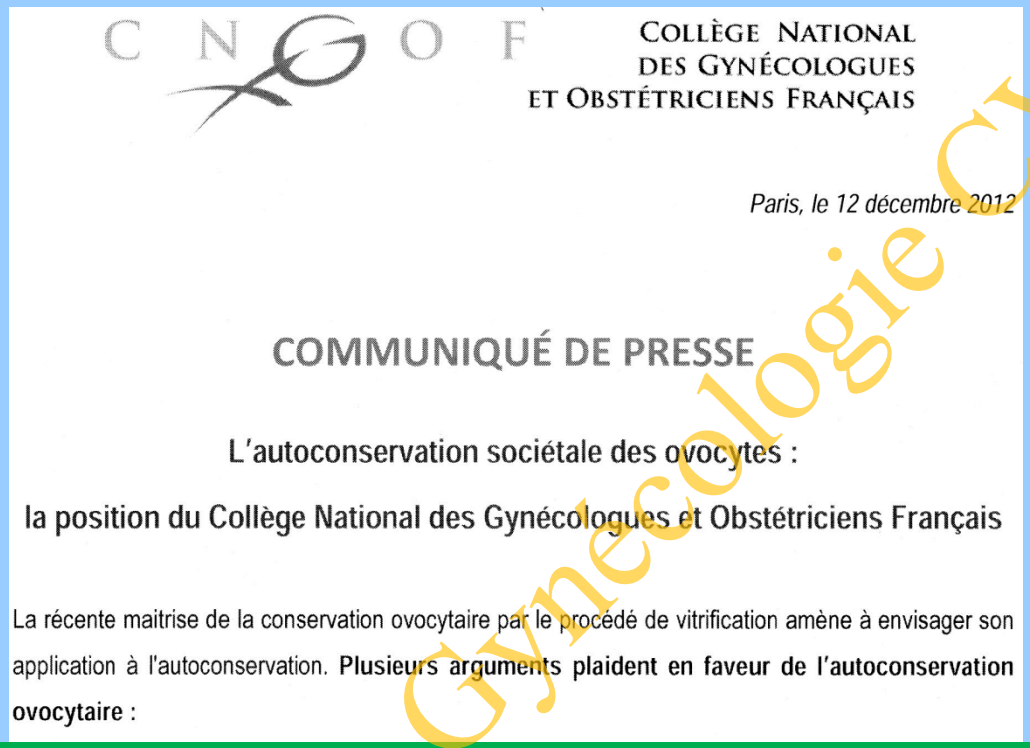
II. – L'article L. 1244-2 du même code est ainsi modifié :

1° Le début de la seconde phrase du premier alinéa est ainsi rédigé : « Le consentement des donneurs et, s'ils font partie d'un couple,... *(le reste sans changement).* » ;

2° Il est ajouté un alinéa ainsi rédigé :

« Lorsqu'il est majeur, le donneur peut ne pas avoir procréé. Il se voit alors proposer le recueil et la conservation d'une partie de ses gamètes ou de ses tissus germinaux en vue d'une éventuelle réalisation ultérieure, à son bénéfice, d'une assistance médicale à la procréation, dans les conditions prévues au titre IV du livre Ier de la deuxième partie. Ce recueil et cette conservation sont subordonnés au consentement du donneur. »

Autoconservation sociétale des ovocytes ?



C N G O F

COLLÈGE NATIONAL
DES GYNÉCOLOGUES
ET OBSTÉTRICIENS FRANÇAIS

Paris, le 12 décembre 2012

COMMUNIQUÉ DE PRESSE

L'autoconservation sociétale des ovocytes :

la position du Collège National des Gynécologues et Obstétriciens Français

La récente maitrise de la conservation ovocytaire par le procédé de vitrification amène à envisager son application à l'autoconservation. **Plusieurs arguments plaident en faveur de l'autoconservation ovocytaire :**

- Seule méthode de traitement de l'infertilité efficace à 40 ans et +
- Autorisée par la loi de juillet 2011 pour raison médical
- Recul constant de l'âge de la maternité
- Autoconservation de convenance possible pour les hommes
- Chantage non acceptable au don d'ovocytes
- Accepté dans de nombreux pays

- Encouragement des grossesses tardives
- Définir un âge limite pour ne pas donner de faux espoirs
- Problème du financement

Voici celle qui a été approuvée par le conseil d'administration à une majorité de plus des deux tiers :

- Âge de conservation : l'autoconservation est optimale avant 35 ans, possible jusqu'à 39 ans (si la réserve ovarienne le permet), mais les femmes doivent être informées qu'au-delà de 35 ans les chances d'obtenir une grossesse diminuent notablement. Il n'est pas souhaitable de faire une autoconservation avant l'âge de 30 ans, sauf indication médicale avérée.
- Âge limite pour reprendre ses ovocytes : optimal avant 45 ans, éventuellement possible entre 45 et 50 ans sous réserve que l'état de santé de la femme ne soit pas incompatible avec le bon déroulement d'une grossesse et que a femme soit dûment informée des risques tant pour elle que pour l'enfant.

Bernard Hédon

05/07/2013

Oocyte banking for anticipated gamete exhaustion (AGE) is a preventive intervention, neither social nor nonmedical

Dominic Stoop [a,*], Fulco van der Veen [b], Michel Deneyer [c], Julie Nekkebroeck [a], Herman Tournaye [a]

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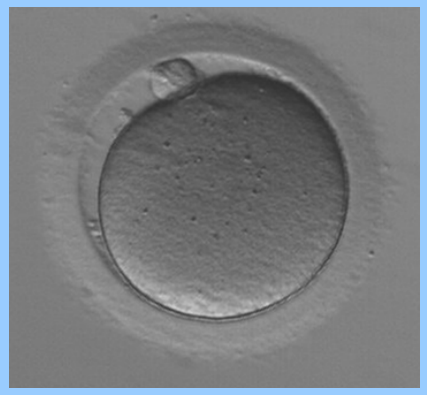
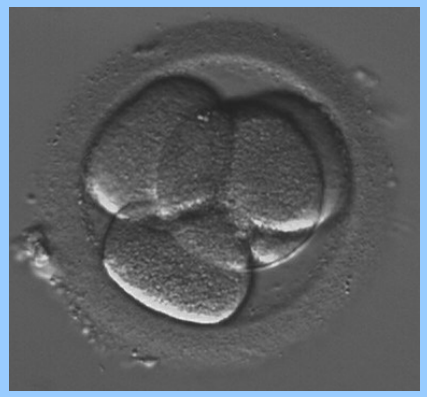
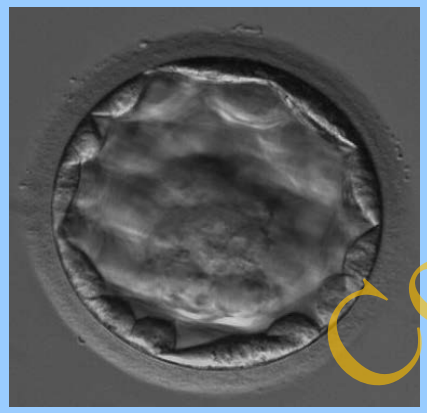
Stoop et al, RBM Online 2014

Conclusions







VITRIFICATION

Résultats +++

Résultats +

Résultats +++

Vitrification

- **Bénéfices**
  - Préservation de la fertilité féminine
  - PEC des couples
- **Evaluations à mener**
  - Stratégies de PEC
  - Etat des enfants nés
- **Phase dynamique**
  - Impact sur la mobilité des couples
  - Accueil d'embryons
- **Coût élevé (X5)**