AUTOMATED VITRIFICATION

Miquel Solé Inarejos, PhD

Hospital Universitario
Dexeus
Barcelona

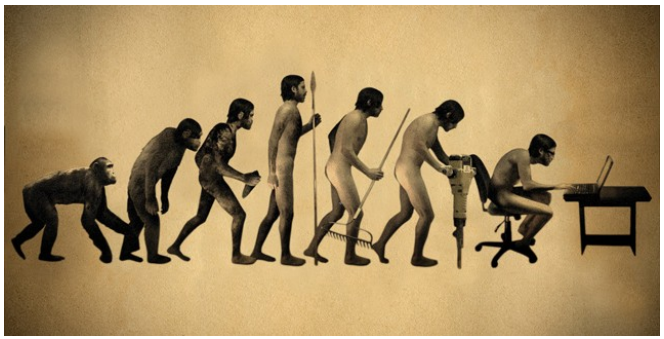
Disclousure

I have the following potential conflict of interest:

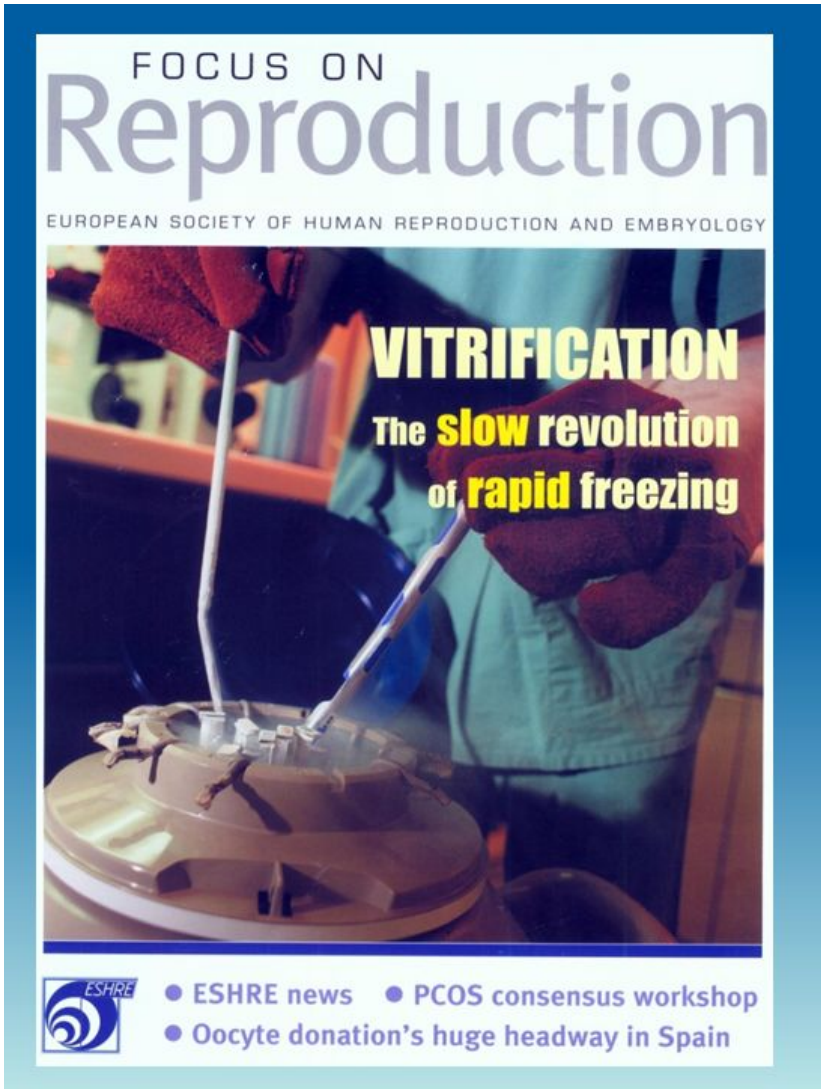
Receipt of research suport, material and media for GAVI system

New technologies

- Great advances in Assisted Reproduction Technologies (ART) in recent years in all areas
  - IVF lab: undisturbed culture (*time-lapse)*
  - PGD lab: CGH, NGS
  - Cryobiology lab: vitrification



Vitrification re-surface



Vitrification becomes really important tool in TRA

Vitrification methodology

- Process requires high concentrations of CPA and high cooling-warming rates
- Initially introduced with cleavage stage embryos (Mukaida *et al.* 1998)
- A little later with oocytes (Kuleshova *et al.* 1999)
- Many modifications in the last 20 years
- Excess of carriers in the market – not all with adequate cooling-warming rates
- Cryotop most commonly used protocol for oocytes and embryos (Kuwayama *et al.* 2005).

Efficiency of vitrification

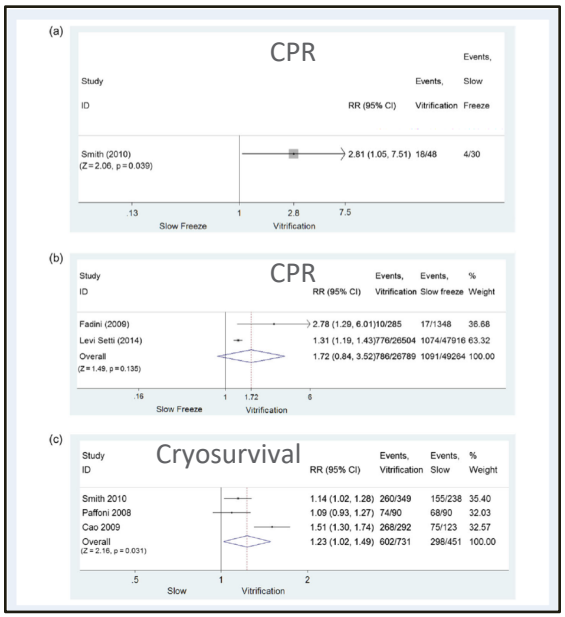
Human Reproduction Update, Vol.23, No.2 pp. 139–155, 2017
Advanced Access publication on November 4, 2016 doi:10.1093/humupd/dmw038

human reproduction update

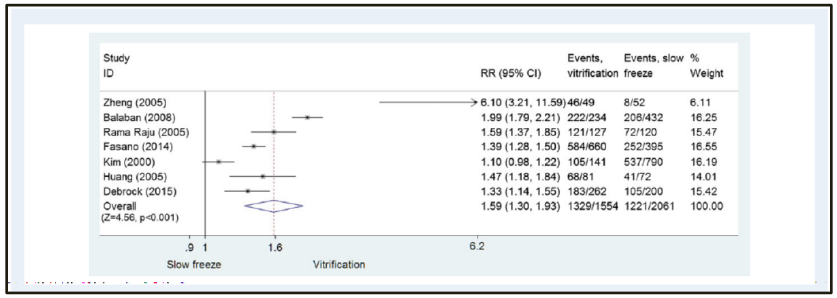
Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance

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

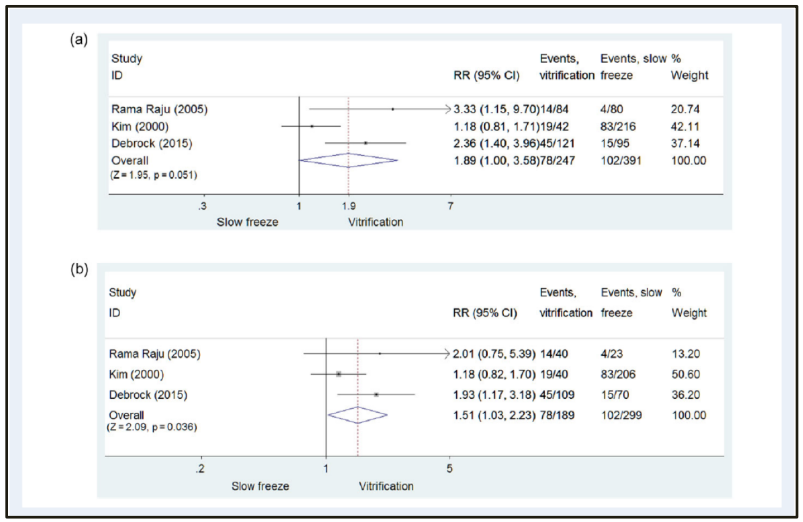
[1]GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, via de Notaris 2b, Rome, Italy [2]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA [3]American Society for Reproductive Medicine, Birmingham, Alabama 35216, USA [4]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA [5]HRP (the UNDP/UNFPA/UNICEF/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction), Geneva, Switzerland (at the time of the study) [6]Population Council, Reproductive Health Programme, New York, USA



Oocyte slow freezing versus vitrification

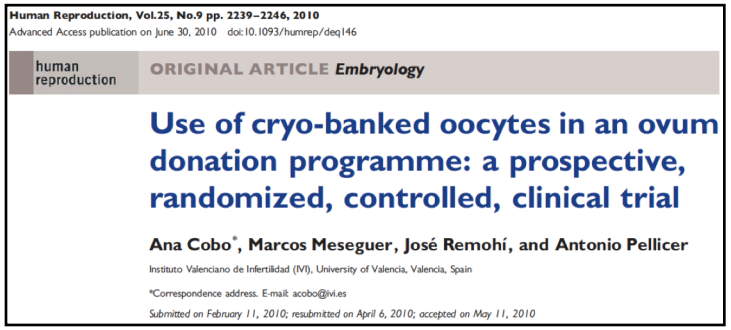


Embryo slow freezing versus vitrification: Cryosurvival



Embryo slow freezing versus vitrification: CPR

Results in oocyte vitrification



Human Reproduction, Vol.25, No.9 pp. 2239–2246, 2010
Advanced Access publication on June 30, 2010 doi:10.1093/humrep/deq146

human reproduction — ORIGINAL ARTICLE *Embryology*

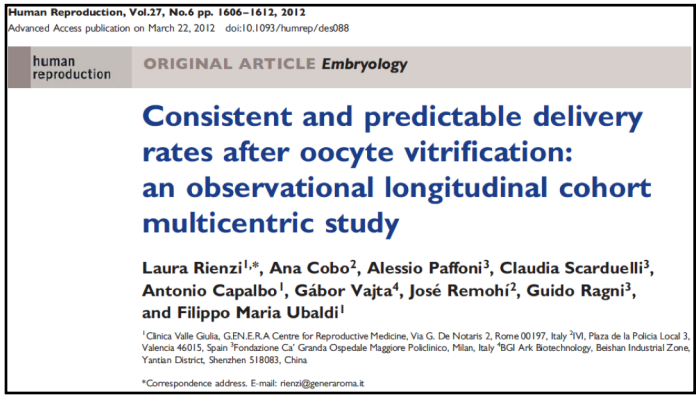
Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial

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

Instituto Valenciano de Infertilidad (IVI), University of Valencia, Valencia, Spain

*Correspondence address. E-mail: acobo@ivi.es

Submitted on February 11, 2010; resubmitted on April 6, 2010; accepted on May 11, 2010



Human Reproduction, Vol.27, No.6 pp. 1606–1612, 2012
Advanced Access publication on March 22, 2012 doi:10.1093/humrep/des088

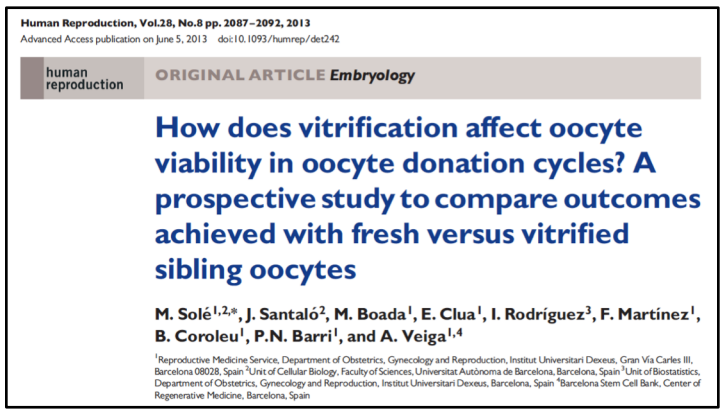
human reproduction — ORIGINAL ARTICLE *Embryology*

Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study

Laura Rienzi[1,*], Ana Cobo[2], Alessio Paffoni[3], Claudia Scarduelli[3], Antonio Capalbo[1], Gábor Vajta[4], José Remohí[2], Guido Ragni[3], and Filippo Maria Ubaldi[1]

[1]Clinica Valle Giulia, G.EN.E.R.A Centre for Reproductive Medicine, Via G. De Notaris 2, Rome 00197, Italy [2]IVI, Plaza de la Policia Local 3, Valencia 46015, Spain [3]Fondazione Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy [4]BGI Ark Biotechnology, Beishan Industrial Zone, Yantian District, Shenzhen 518083, China

*Correspondence address. E-mail: rienzi@generaroma.it



Human Reproduction, Vol.28, No.8 pp. 2087–2092, 2013
Advanced Access publication on June 5, 2013 doi:10.1093/humrep/det242

human reproduction — ORIGINAL ARTICLE *Embryology*

How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes

M. Solé[1,2,*], J. Santaló[2], M. Boada[1], E. Clua[1], I. Rodríguez[3], F. Martínez[1], B. Coroleu[1], P.N. Barri[1], and A. Veiga[1,4]

[1]Reproductive Medicine Service, Department of Obstetrics, Gynecology and Reproduction, Institut Universitari Dexeus, Gran Via Carles III, Barcelona 08028, Spain [2]Unit of Cellular Biology, Faculty of Sciences, Universitat Autònoma de Barcelona, Barcelona, Spain [3]Unit of Biostatistics, Department of Obstetrics, Gynecology and Reproduction, Institut Universitari Dexeus, Barcelona, Spain [4]Barcelona Stem Cell Bank, Center of Regenerative Medicine, Barcelona, Spain

- Fertilization rate
- Embryo quality
- Clinical pregnancy rate
- Live birth rate

Oocyte donation programme: Clinical pregnancy rate

Number of cycles: 3292 (2012-2018)

Inseminated oocytes	Fresh oocytes n= 1106	Vitrified oocytes n= 633
1-4	34,6%	35.3%
5-7	46.2%	44.5%
8-12	56.4%	53.2%
>12	60.4%	57.1%

≠ 2-3%

A critical look at our results: can we improve them?

Oocyte donation programme:

SURVIVAL RATE: 80-85%

- Survival rate: 82.7% (9473/11448)
- Oocyte donation with <50% of survival rate: 4.5%

Fertility preservation for social reasons:

- Survival rate: 80.6% (82/98)
- Pregnancy rate: 50% (5/10)

Fertility preservation for medical reasons:

- Survival rate: 85.1% (74/87)
- Pregnancy rate: 62.5% (5/8)

PGD programme: oocyte accumulation:

- Survival rate: 81.6%
- Biopsiable embryos on D3 (vitrified vs fresh): 75.6% vs 93.8%

Data from Society for Assisted Reproduction Technology (SART)

Kushnir *et al. Journal of Ovarian Research* (2018) 11:2
DOI 10.1186/s13048-017-0378-4

Journal of Ovarian Research

RESEARCH Open Access

New national outcome data on fresh versus cryopreserved donor oocytes

Vitaly A. Kushnir[1,2*], Sarah K. Darmon[1], David H. Barad[1,3] and Norbert Gleicher[1,3,4,5]

Table 1 Fresh and Cryopreserved Donor Oocyte cycles reported to Society for Assisted Reproductive Technology, 2013–2015

	Fresh Donor Oocytes			Cryopreserved Donor Oocytes		
Year(s)	2013	2014	2015[a]	2013	2014	2015[a]
Number of cycles	8921	6929	5982	2227	2886	3215
Average number of transferred embryos	1.7	1.6	1.6	1.6	1.6	1.6
Cancelled Cycles (%)	11.7	7.0	9.1	8.5	12.4	15.0

[a]2015 data were calculated from a preliminary report distributed by SART

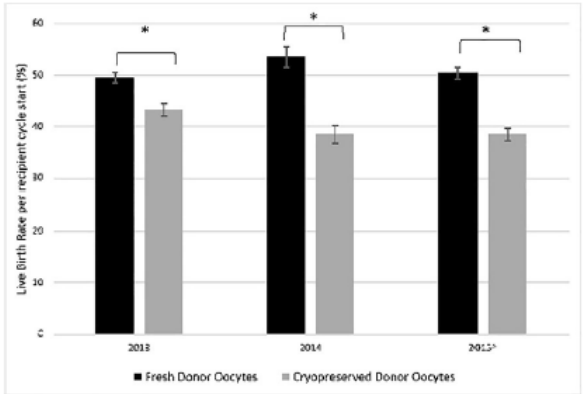


Fig. 1 Live birth rates with fresh versus cryopreserved donated oocytes, 2013–2015. * $P < 0.001$; Live birth rates were compared using the two-tailed Fisher's exact test and Wilson confidence interval for binomial proportions. ^ 2015 data were calculated from a preliminary report distributed by SART

Lack of reproducibility

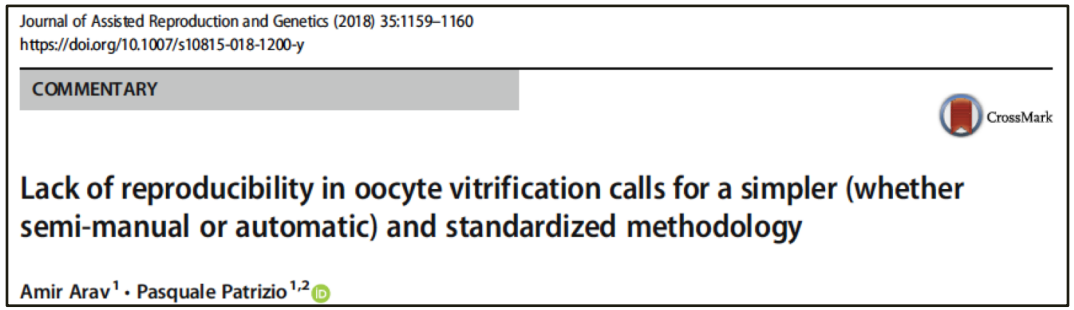
Journal of Assisted Reproduction and Genetics (2018) 35:1157–1158
https://doi.org/10.1007/s10815-018-1184-7

COMMENTARY

CrossMark

Issues related to human oocyte vitrification: a consideration of the facts

Samer Tannus[1] · Michael-Haim Dahan[2,3] · Justin Tan[4] · Seang-Lin Tan[2,3]



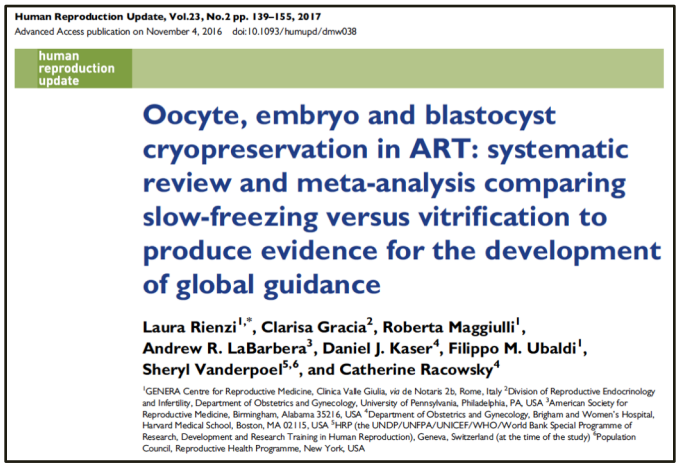
Journal of Assisted Reproduction and Genetics (2018) 35:1159–1160
https://doi.org/10.1007/s10815-018-1200-y

COMMENTARY

CrossMark

Lack of reproducibility in oocyte vitrification calls for a simpler (whether semi-manual or automatic) and standardized methodology

Amir Arav[1] · Pasquale Patrizio[1,2]



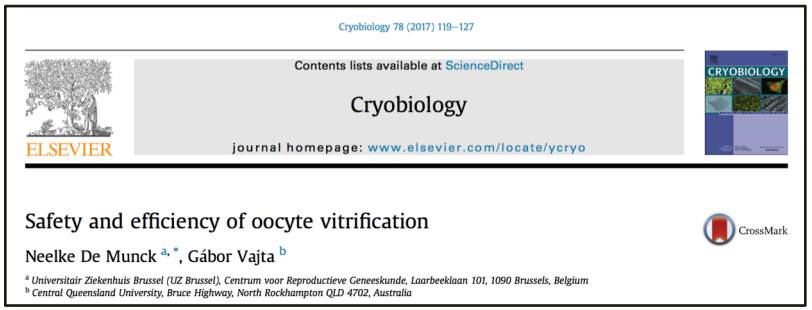
Human Reproduction Update, Vol.23, No.2 pp. 139–155, 2017
Advanced Access publication on November 4, 2016 doi:10.1093/humupd/dmw038

human reproduction update

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

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

[1]GENERA Centre for Reproductive Medicine, Clinica Valle Giulia, via de Notaris 2b, Rome, Italy [2]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA, USA [3]American Society for Reproductive Medicine, Birmingham, Alabama 35216, USA [4]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA [5]HRP (the UNDP/UNFPA/UNICEF/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction), Geneva, Switzerland (at the time of the study) [6]Population Council, Reproductive Health Programme, New York, USA



Cryobiology 78 (2017) 119–127

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Safety and efficiency of oocyte vitrification

CrossMark

Neelke De Munck [a,*], Gábor Vajta [b]

[a] Universitair Ziekenhuis Brussel (UZ Brussel), Centrum voor Reproductieve Geneeskunde, Laarbeeklaan 101, 1090 Brussels, Belgium
[b] Central Queensland University, Bruce Highway, North Rockhampton QLD 4702, Australia

Vitrification: the case for automation

- Totally manual process
  - ➢ High level of specialisation and experience
  - ➢ Incubation timings with cryoprotectants are critical
  - ➢ Variability in results

PRO	CONS
- Standardises process quality - Saves time - Avoids learning curve	- Warming procedure still manual - Initial financial investment

How can we improve quality of the process?

- Efficiency and consistency need to be improved and only an automated vitrification system can achieve this.
- Ideal device should enable:
  - Easy and safe handling
  - Time and temperature control
  - Minimize human manipulation: standarization of volumes and timings
  - Reduce operator time required per procedure
  - Closed systems ==> avoid risk of contamination
  - Automatic labelling-tracking system
  - Automated sample transfer to storage containers.

Microfluidic technologies

Molecular Human Reproduction, Vol.23, No.4 pp. 257–268, 2017
Advanced Access publication on February 2, 2017 doi:10.1093/molehr/gaw076

molecular human reproduction

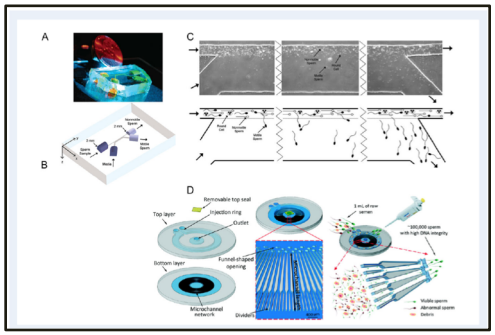
NEW RESEARCH HORIZON *Review*

Application of microfluidic technologies to human assisted reproduction

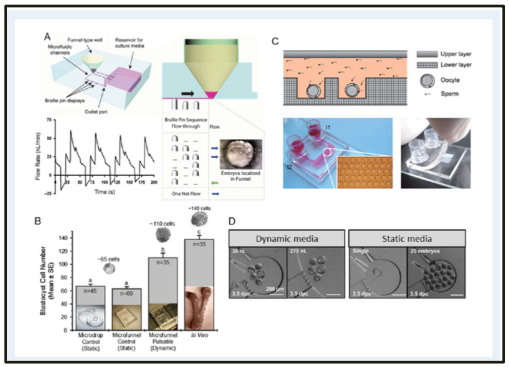
Gary D. Smith[1,*] and Shuichi Takayama[2]

[1]Departments of Obstetrics and Gynecology, Physiology and Urology, University of Michigan, 6428 Medical Sciences I, 1301 E Catherine Street, Ann Arbor, MI 48108-1649, USA [2]Departments of Biomedical Engineering and Macromolecular Science and Engineering, University of Michigan, Ann Arbor, MI, USA

- Microfluidics considered both science and technology.
- At least two characteristics of microfluidics
  - Mechanical
  - Biochemical
- Controlled cryoprotectant exposure

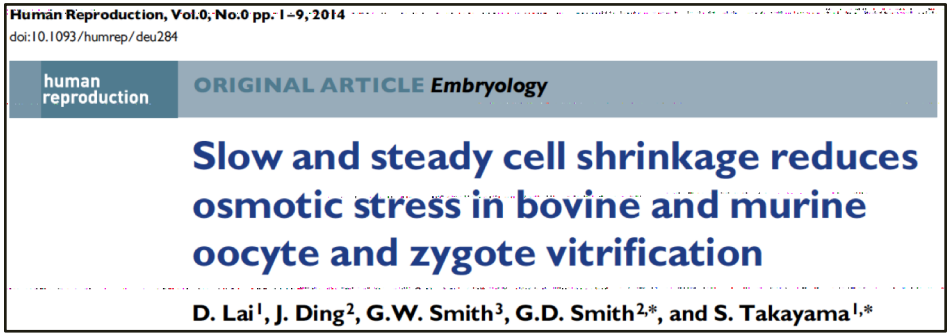


Microfluidic sperm sorter



Embryo culture with microfluids

Microfluidic technologies in vitrification

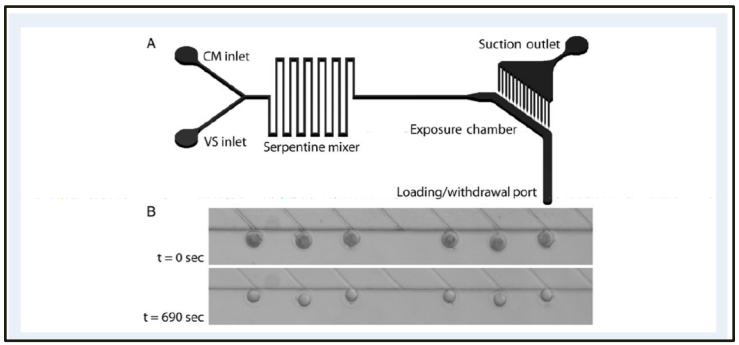

Human Reproduction, Vol.0, No.0 pp. 1–9, 2014
doi:10.1093/humrep/deu284

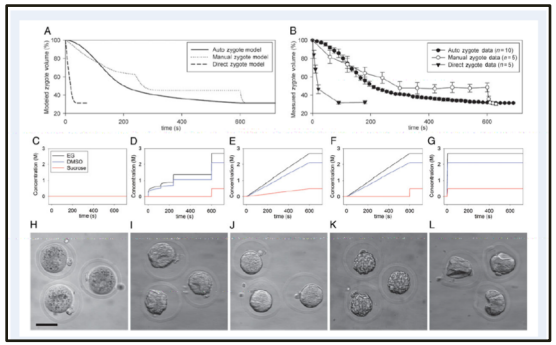
human reproduction

ORIGINAL ARTICLE *Embryology*

Slow and steady cell shrinkage reduces osmotic stress in bovine and murine oocyte and zygote vitrification

D. Lai[1], J. Ding[2], G.W. Smith[3], G.D. Smith[2,*], and S. Takayama[1,*]





- Already used as cryoprotectant exchange system to generate automated, continuous and gradual cryoprotectants addition.
- Developed user-friendly microfluidic CPA exchange system.
- May also be advantageous to an automated warming process

First digital microfluidic device

OPEN ACCESS Freely available online

PLOS | ONE

Digital Microfluidic Processing of Mammalian Embryos for Vitrification

Derek G. Pyne[1], Jun Liu[1], Mohamed Abdelgawad[2]*, Yu Sun[1]*

1 Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario, Canada, 2 Department of Mechanical Engineering, Assiut University, Assiut, Egypt

September 2014 | Volume 9 | Issue 9 | e108128

Table 1. Summary of vitrification results.

	Survival Rate	Development Rate
control (non-vitrified)	100% (14/14)	93% (13/14)
manual	73% (11/15)	91% (10/11)
DMF Chip	77% (10/13)	90% (9/10)

doi:10.1371/journal.pone.0108128.t001

- First digital microfluidic device showing feasibility to perform automated embryo processing for vitrification
- Low number but promising results.

Becomes a reality: GAVI™ first automated vitrification instrument

Human Reproduction, Vol.29, No.11 pp. 2431–2438, 2014
Advanced Access publication on August 27, 2014 doi:10.1093/humrep/deu214

human reproduction

ORIGINAL ARTICLE ***Embryology***

Embryo vitrification using a novel semi-automated closed system yields *in vitro* outcomes equivalent to the manual Cryotop method

Tammie K. Roy[1,*], Susanna Brandi[1], Naomi M. Tappe[1], Cara K. Bradley[1], Eduardo Vom[2], Chester Henderson[2], Craig Lewis[2], Kristy Battista[2], Ben Hobbs[2], Simon Hobbs[2], John Syer[2], Sam R. Lanyon[1], Sacha M. Dopheide[2], Teija T. Peura[1], Steven J. McArthur[3], Mark C. Bowman[3], and Tomas Stojanov[3]

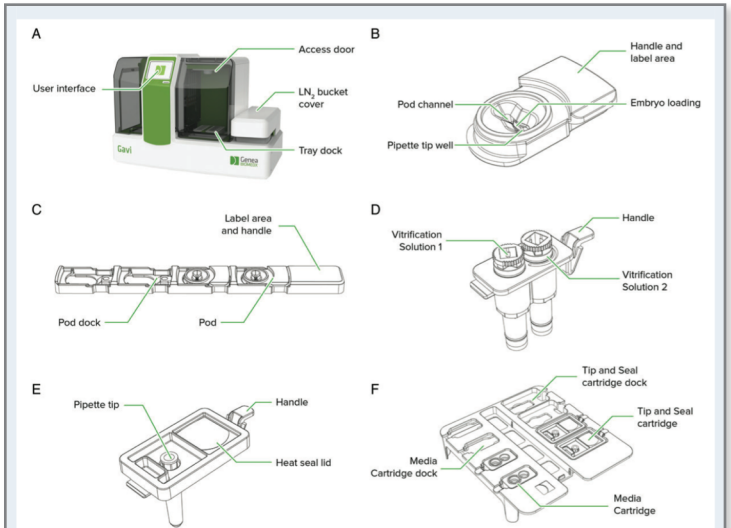
[1]Genea Biomedx, 321 Kent Street, Sydney, NSW 2000, Australia [2]Planet Innovation, 81-89 Cotham Road, Kew, VIC 3101, Australia [3]Genea, 321 Kent Street, Sydney, NSW 2000, Australia

*Correspondence address. E-mail: tammie.roy@geneabiomedx.com.au

Table V Evaluation of the Gavi system for human blastocysts.

Treatment	#	Recovered	Survived	Re-expanded	24 h		48 h	
					Fully hatched blastocyst	Hatching/expanded blastocyst	Fully hatched blastocyst	Hatching blastocyst
Cryotop	13	13 (100%)	10 (77%)	7/10 (70%)[a]	1 (8%)	9 (69%)	2 (15%)	3 (23%)
Gavi	23	23 (100%)	21 (91%)	14/18 (78%)[a]	4 (17%)	12 (52%)	4 (17%)	6 (26%)

Outcomes for human blastocysts vitrified after automated processing with the Gavi system, as compared with the manual Cryotop method. Embryo classifications at 24 and 48 h were restricted to grade I and grade II embryos.
[a]Not all embryos were assessed for re-expansion.



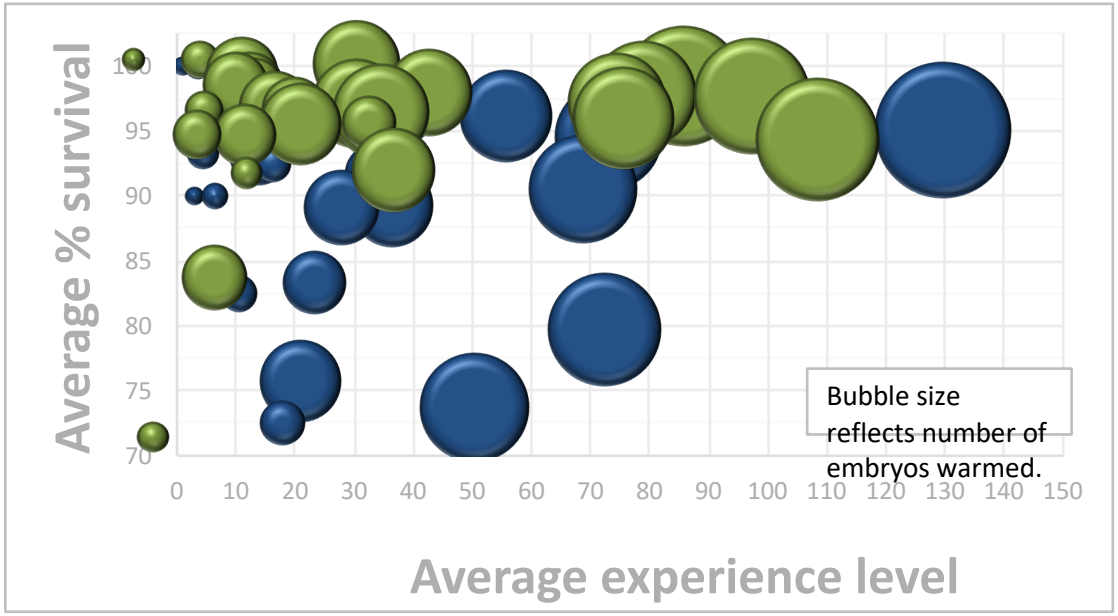
Supplementary Figure S1 Components of the Gavi system. (**A**) Gavi instrument. (**B**) Pod. (**C**) Cassette. (**D**) Media Cartridge. (**E**) Tip and Seal. (**F**) Tray. All figures are of Alpha prototypes and are schematics with the exception of (A) which is a CAD (computer-aided design) drawing.

GAVI™: first automated vitrification instrument

- Semi-automated system
  - Plunge in liquid nitrogen
  - Warming procedure
- GAVI provides automated cryoprotector exposure and sealing device.
- Elimination of variability:
  - During exposure to cryoprotectants:
    - Temperature
    - Volume (equilibration media)
    - Microfluidic technology
    - Constant volume of vitrification media into device

Variability also with embryos

- Recovered embryo survival by scientist – Gavi vs. Cryotop



Values	GAVI	Cryotop
Embryos warmed	361	361
# clinics	6	8
# freeze scientists	28	25
% recovered	98.30%	99.70%
Average survival %	96.50%	88.10%
Embryo with 100% survival	62.0%*	43.3%*
Embryos with 50% survival	0.80%	7.20%

* P value <0.0001

GAVI vs Cryotop: survival rate of human blastocysts

- Summary of survival rate outcomes

	Gavi	Cryotop
# vitrified	907	932
# warmed (for ET)	270	288
# recovered	267 (98.9%)	288 (100%)
# initial survival >75% (of warmed)	265 (98.1%)	284 (98.6%)
Average initial survival % (of warmed)	95.9%	96.4%
# initial survival 100%	157	145
% initial survival 100%	58.1%*	50.3%*

Data on file (QRTV224_08)

*p=<0.05

Clinically vitrified human blastocysts: implantation and ongoing pregnancy rates

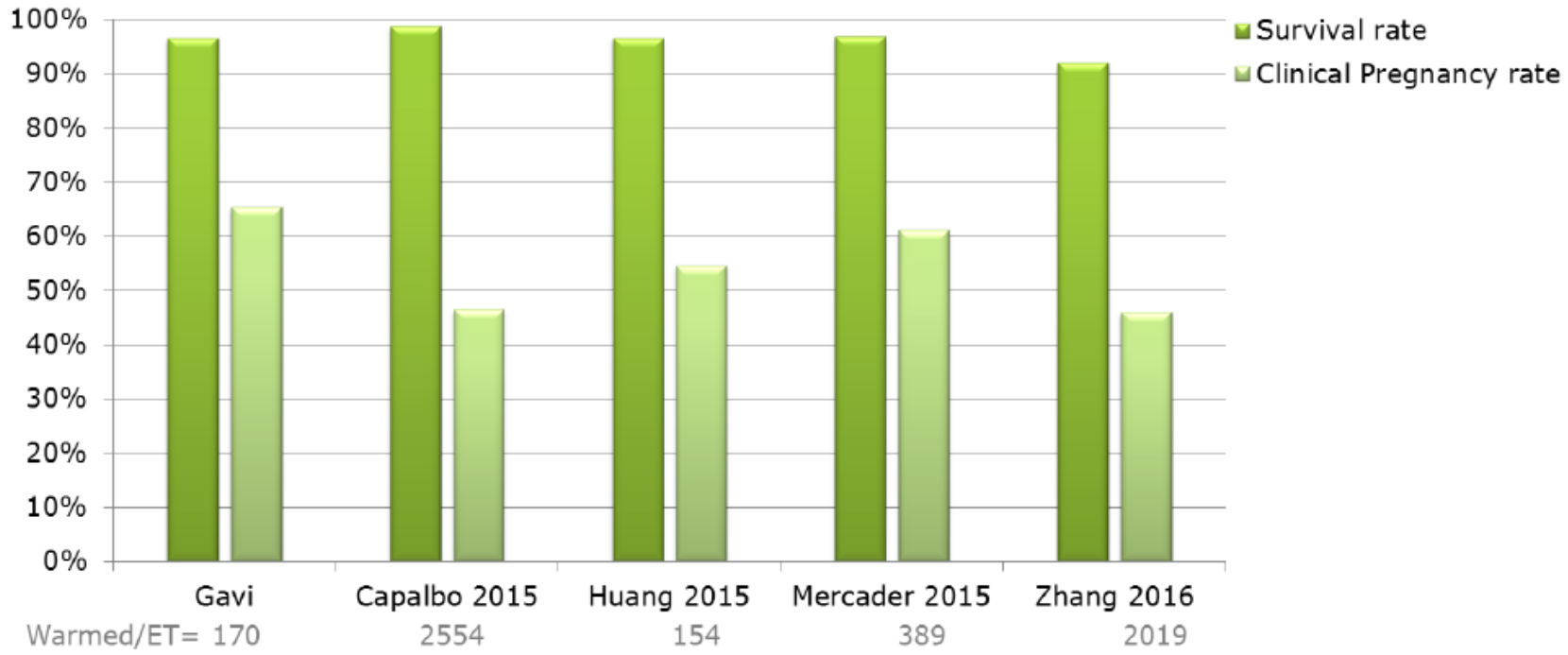
- Summary of embryo transfer and pregnancy outcomes

	Gavi	Cryotop®
Average of Age	36.00	35.99
# ETs	265	284
Sum of #ETd	265	284
# + βhCG	183	179
# FH pregnancies	151	156
Sum of FHs	154	158
% + βhCG	69.1%	63.0%
% FH Pregnancies	57.0%	54.9%
Conversion + βhCG to FH	82.5%	87.2%
Loss +βhCG to FH	17.5%	12.8%

Data on file (QRTV224_08)

Comparison of clinical outcomes from GAVI other publications

- Results after vitrification using Gavi versus after Cryotop vitrification



Data on file (QRTV224_08)

First results with GAVI in human biopsied blastocysts

PGD program I.U. Dexeus

Blastocysts biopsy and CGH

→ CRYOTOP VITRIFICATION

36 ABNORMAL blastocysts

→ WARMING

0/36 Lost embryos

2/36 No survival

2/36 No re-expansion

32 Survival and re-expanded blastocysts (**88.9%**)

VALIDATION

→ GAVI VITRIFICATION/WARMING

0/32 Lost embryos

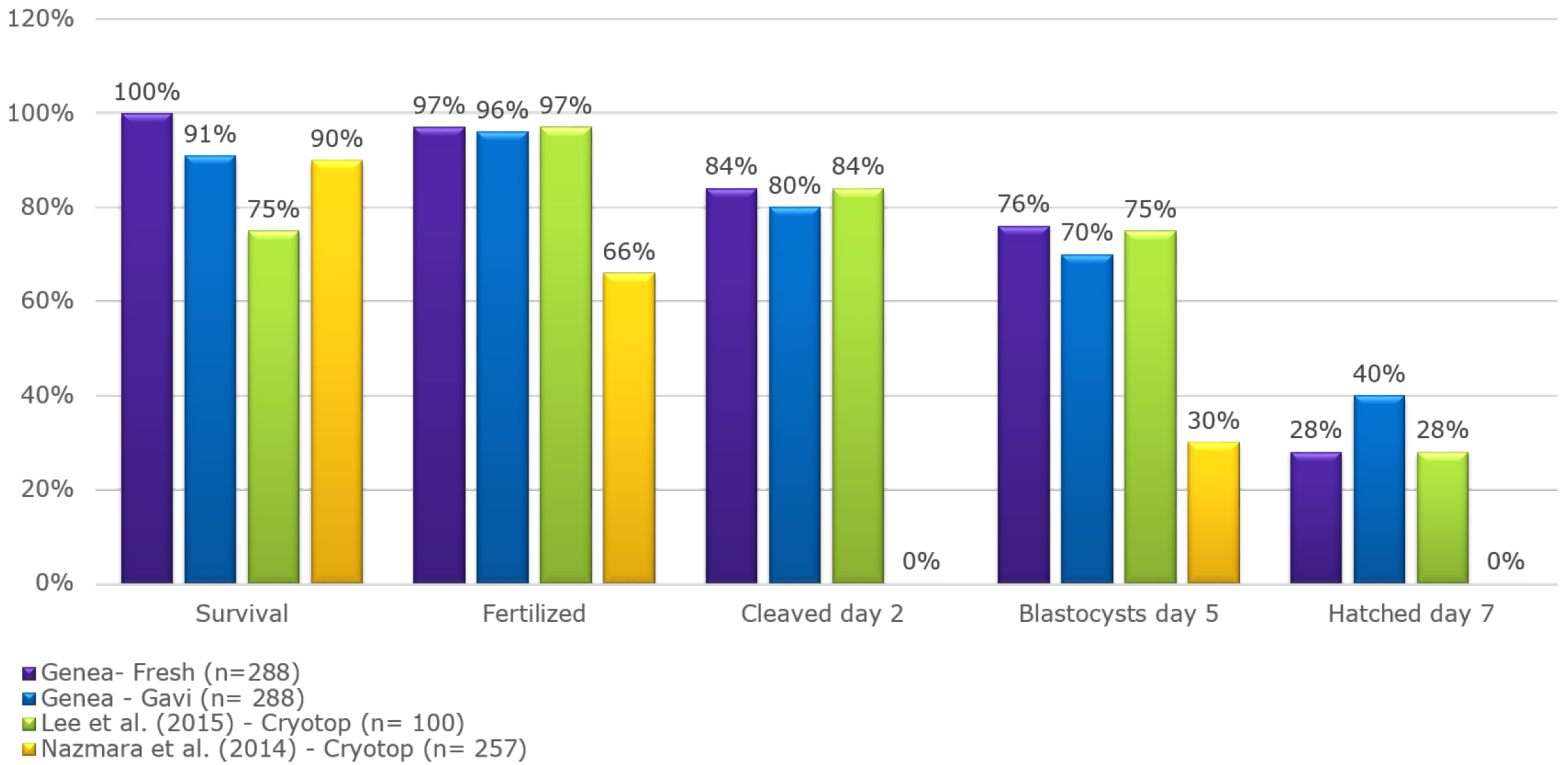
1/32 No survival

3/32 No re-expansion

28 Survival and re-expanded blastocysts (**87.5%**)

GAVI: mouse oocytes fresh and vitrified with different vitrification devices

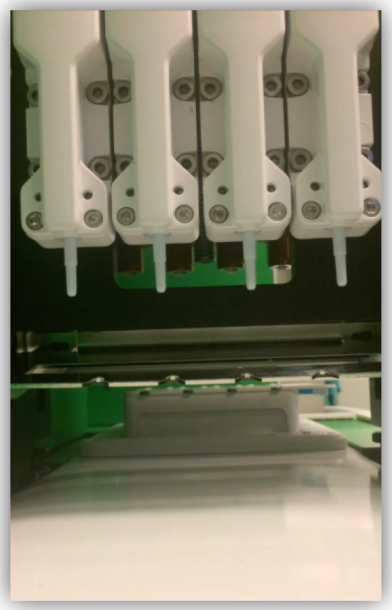
- Survival rate and embryo development



Source:Lee 2015, Mazmara 2014, Brandi S[1], Ho PPY[1], Anastasi M[2], Roy TK[1]– Genea at Kent St (Sydney, Australia) site, publication in process of submission.

Oocyte protocol validation: H. U. Dexeus

- Oocyte donation cycles
- Vitrified oocytes ≥ 8 MII
- Cryotop®: ≥4 MII
- GAVI™: ≥4 MII
- All oocytes warmed in each recipient cycle
- Embryos transferred on day 3
- Blind embryo selection using ASEBIR score (Cryotop vs GAVI)
- Embryo vitrification on days 3 or 5



Oocyte protocol validation: H. U. Dexeus

	Cryotop	GAVI	Total
Oocyte donation cycles (n)	23	23	23
Vitrified oocytes (n)	147	155	302
Recipient cycles (n)	13	13	13
Warmed oocytes (n)	63	73	136
Survival (%)	76.2	76,7	76.5
Fertilization (%)	73	76,8	75.0
Ongoing embryos (%)	54.3	53.5	53.8

ASRM 2017

- First 2 births with GAVI worldwide

New devices coming… SARAH

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https://doi.org/10.1007/s10815-018-1210-9

TECHNOLOGICAL INNOVATIONS

A new, simple, automatic vitrification device: preliminary results with murine and bovine oocytes and embryos

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	Oocytes	8-cell embryo	Blastocysts	Control (fresh Embryos)
Number	40	35	165	42
Survival (%)	38/40 (95)	33/35 (94)	160/165 (97)	–
Viability (%)	38/38 (100)	–	–	–
Blastocysts (%)	–	33/35 (94)	–	42/42 (100)
Hatching (%)	–	28/35 (80)	135/165 (81)	32/42 (76)

Conclusions

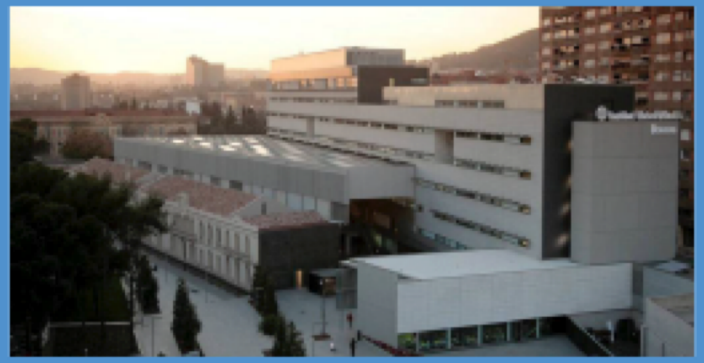
- Vitrification optimization allows large range of reproductive strategies
- Very good results but margin for improvement
- Manual technique => high results variability especially with oocytes
- Urgent need for vitrification and warming optimization ideally automated with minimal reliance on manual techniques
- First microfluidics vitrification applications show promising results

Conclusions

- GAVI can minimise small variations between different manual vitrification processes.
- Demonstrated efficiency with blastocysts comparable to the most efficient manual techniques
- Results appear promising for oocyte vitrification with GAVI™
- Vitrification automation now a real option for laboratories
- However considerable financial investment required and actual implementations still limited

Ayer, hoy y siempre

Dexeus mujer



Thank you for your attention

Gràcies per la seva atenció

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