

Vitrification:

"Robots" versus Human

Comparing automated vitrification outcomes

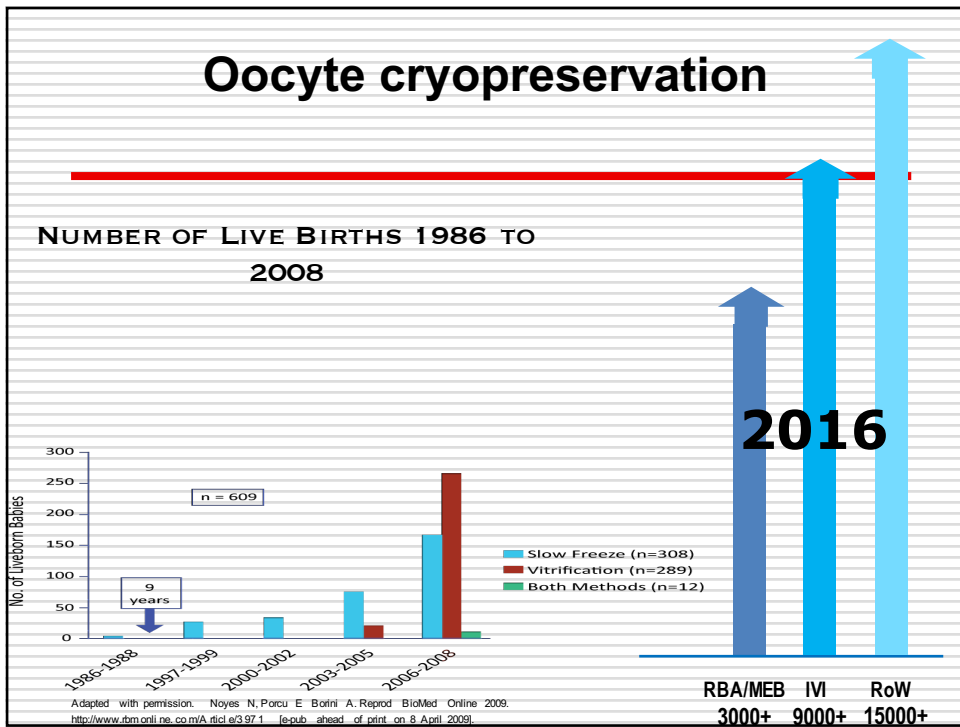
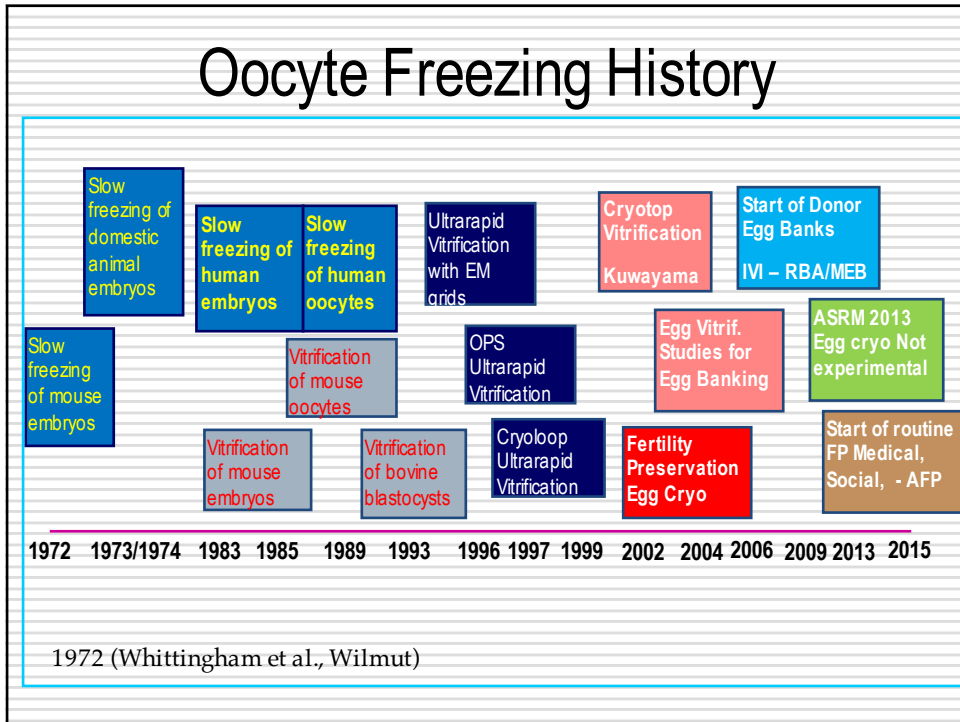
The 5th World congress of the
INTERNATIONAL SOCIETY FOR FERTILITY PRESERVATION
Vienna, Austria | November 16-18, 2017

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Disclosure

Direct stockholder:	My Egg Bank/Prelude
Scientific Advisory Board:	Cooper Surgical/Origio
Paid Consultant:	EMD-Serono
Speakers bureau:	MERCK MSD



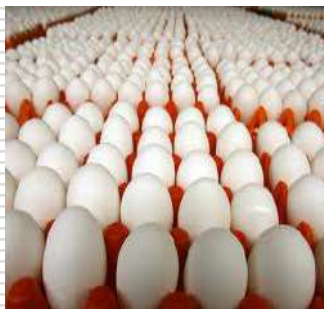
Why to cryopreserve eggs?

- Government restrictions / legislation
- Fertility preservation
 - Medical
 - Social
- Emergency cycle management
 - Failure to obtain sperm
- Elective cycle management
 - Ethical/Religious reasons
 - Ovarian hyperstimulation syndrome (OHSS)
- Donor egg banking

Need for an efficient technique

CHALLENGE: **Efficiency**

100-150 CRYO EGGS → 1 PREGNANCY
(= 10-15 PATIENTS / TRIALS)



1986-2006
LOW FREEZING



Need for an efficient technique

CHALLENGE: **Solved today?**

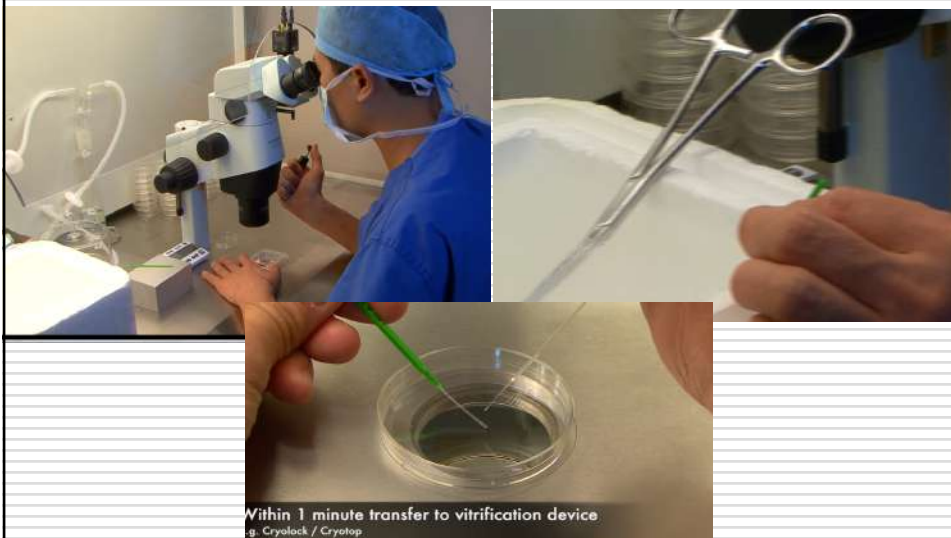
4-5 CRYO EGGS → 1 PREGNANCY



AFTER 2007

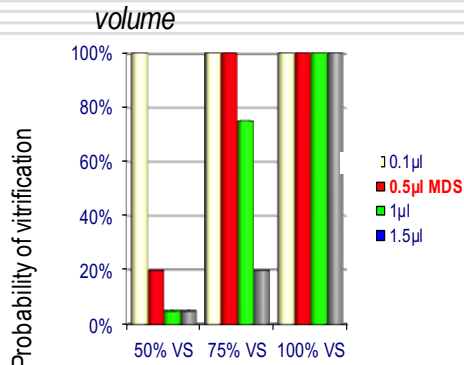
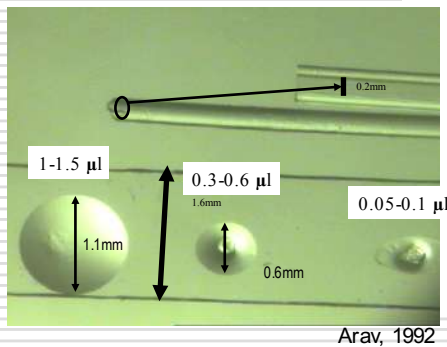


Handling and results with “traditional” Vitrification



The effect of volume on the probability of vitrification

Probability of vitrification = $\frac{\text{cooling and warming rate}}{\text{viscosity}}$

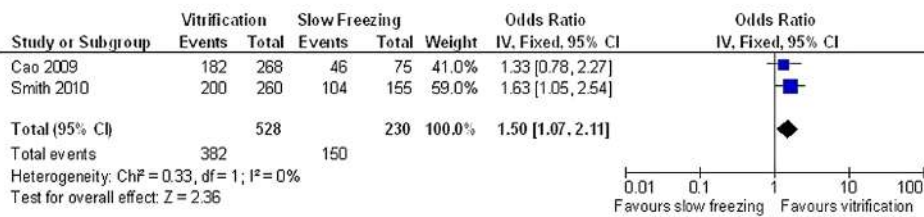


The use of minimum volume allows a reduction in the CPA concentration.

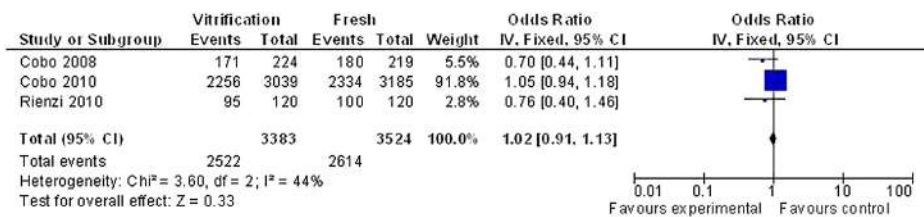
Review of literature: meta-analysis of randomized controlled trials

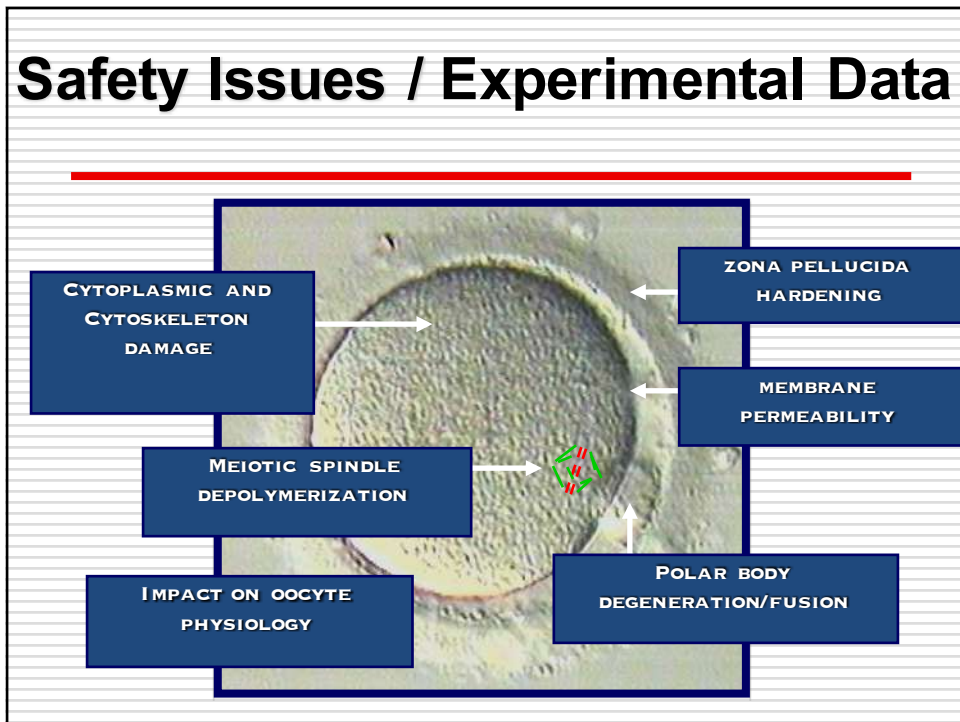
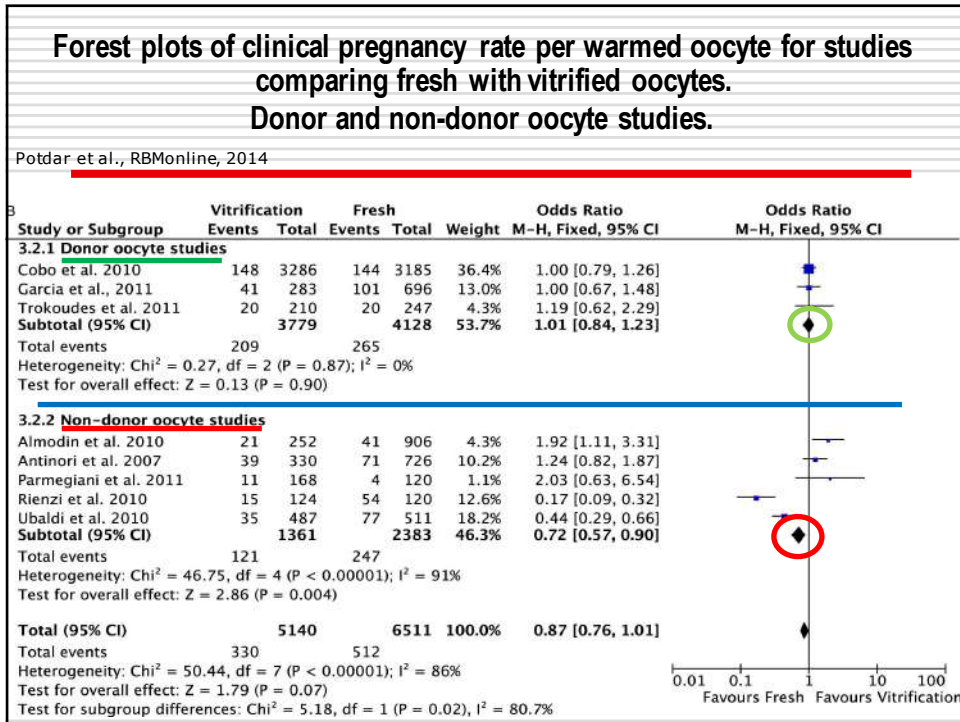
Cobo and Diaz, FS 2011

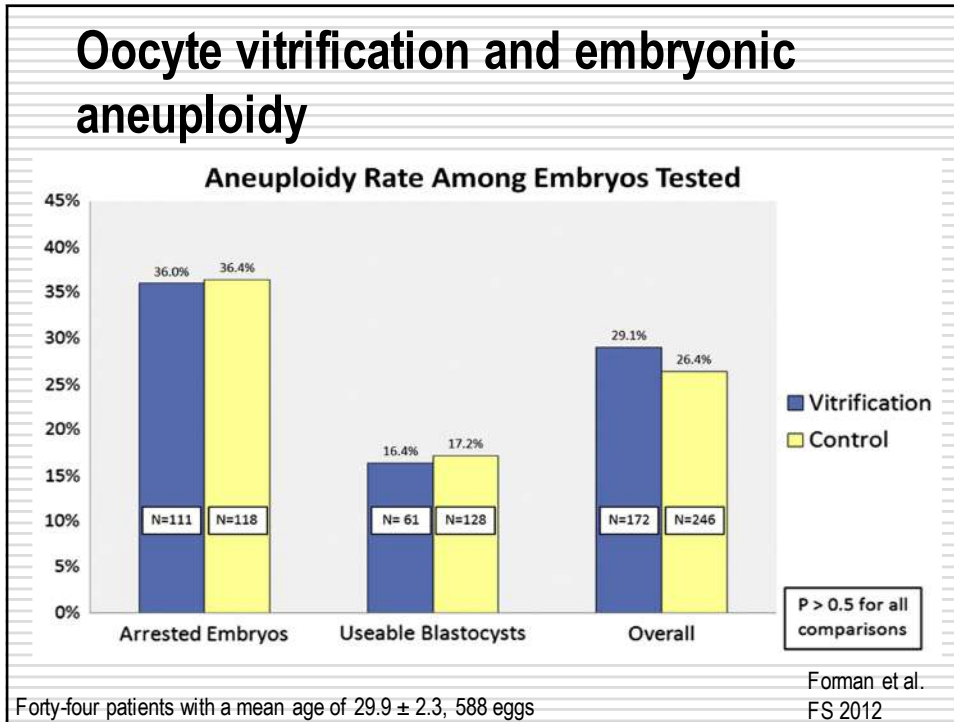
A Vitrification vs. Slow freezing. Fixed effects model



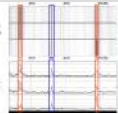
B Vitrification vs. Fresh oocytes. Fixed effects model



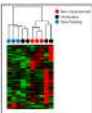




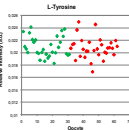
Safety Issues / Experimental Data



Slow freezing has a dramatic effect on cell physiology whereas vitrification appears to have limited effect.
Gardner et.al. Theriogenology 2007



Effects of slow freezing (DNA repair, cell cycle and chromosomal stability) are more deleterious for biological functions than those observed for vitrification (ubiquitination).
Monzo et.al. Hum Reprod 2011



Oocyte vitrification does not disturb embryonic metabolomic profiles.
Dominguez, Fertil Steril 2013

Slide courtesy of Dr. Cobo

Efficiency: Results

Efficiency: RESULTS

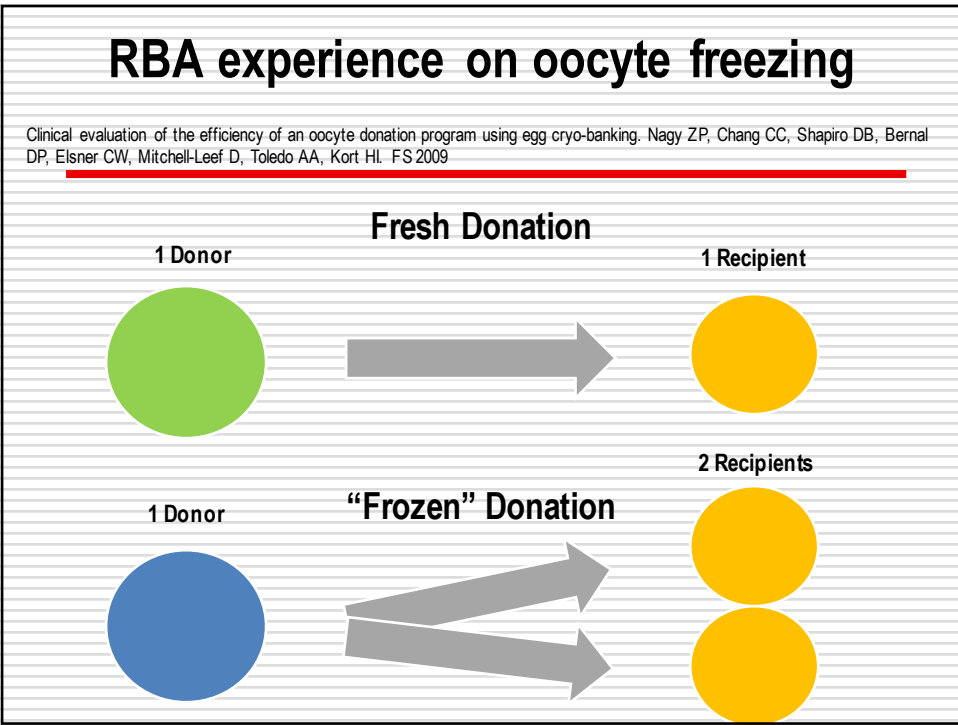
Donor Egg Banking

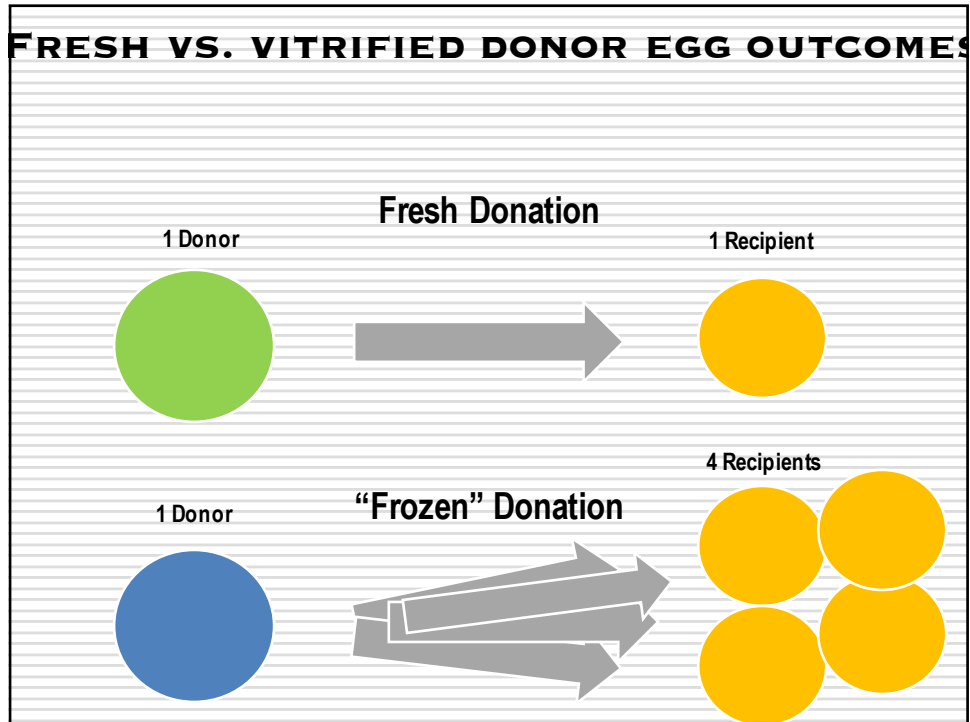
RBA experience on oocyte freezing

Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, Toledo AA, Kort HI. FS 2009

	Cryo Egg	Fresh Egg
Recipients	20	10
M2 Egg/Pat	7.7	31.7
Es for ET (x)	47 (2.4)	19 (2.1)
Es for Cryo (x)	31 (1.5)	141 (16)
PR (IR)	75% (54%)	56% (47%)
Cumulative PR	85%	78%
Recipients Pregnant	17	7

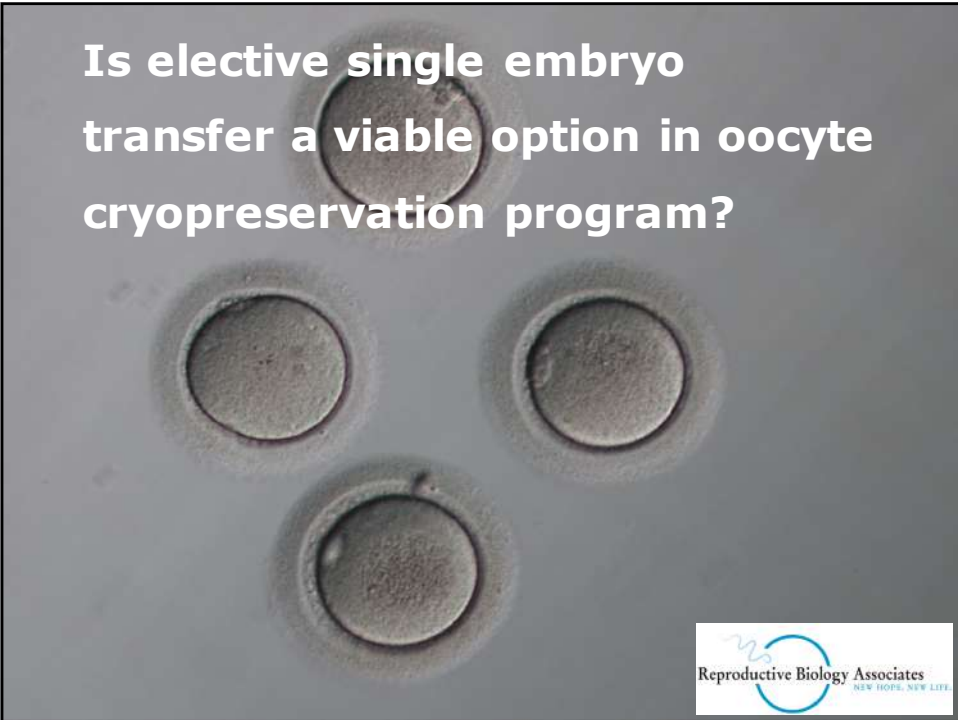
Nagy et al, 2009





MEB experience on donor egg banking

-
- Donation cycles 1,035
 - M2 vitrified 23,060 (22.3/Don.)
 - Recipient cycles 3,424
 - M2 Warmed 21,462 (6.3/Rec.)
 - Survival 88%
 - Fertilization 78%
 - Pregnancy (clinical) 52%



eSET vs. DET after egg vitrification Results

	eSET	eDET	Non-eDET
Number of Recipients	98	109	233
Implanted Embryos	51 (52%)	112 (51%)	171 (37.5%)
Clinical Pregnancies	51 (52%)	79 (72.4%)*	121 (51.9%)
Multiple pregnancies	0 (0%)*	40 (50.6%)	37 (30%)

*P<0.05



Efficiency: RESULTS

ONCE VITRIFIED, TWICE VITRIFIED...



Vitrified Embryos From Vitrified Eggs ("Double Vitrification")

100 patients (Cryo Egg Bank)	<u>Cryo Embryo</u>
Number of warmed embryos	190
Survived	189 (99%)
No of Es for ET (x)	176 (1.8)*
Pregnancies (Clinical)	53 (53%)
Implantation / FCA	68 (39%)
Miscarriages	12
Live births (limited data)	33
Girls	15
Boys	18

* Four of these embryos were biopsied in the first cycle, then vitrified

Transporting vitrified oocytes (embryos)?

IN-HOUSE DONORS VS OUTSIDE (SHIPPED EGGS) LOCATION DONORS

	IN HOUSE DONORS (vit. Eggs – No-Transport)	OUTSIDE DONORS (Transported vit. Eggs)
#CYCLES	1175	63
#DONOR CYCLES	444	52
DONOR AVG. AGE SD	25.8 2.8	25.8 3.1
PATIENT AVG. AGE \pm SD	40.9 \pm 4.71	40.6 \pm 3.9
AVG. # MII WARMED SD*	6.2 \pm 1.85	7.0 \pm 1.0
SURVIVAL RATE	91.0%	89.4%
FERTILIZATION RATE	85.0%	83.8%
BLASTOCYSTS RATE	66.2%	63.0%
AVG. # EMBRYOS TRANSFERRED*	1.5 \pm 0.54	1.2 \pm 0.4
IMPLANTATION RATE	51.3%	48.0%
CLINICAL PREGNANCY RATE	61.6%	57.1%

*P <0.5

Efficiency: Results

Autologous cycles

Outcomes from fresh and warming cycles in patients who choose to electively cryopreserve part of their oocytes		
RBA, 2013	FRESH CYCLE	WARMING CYCLE
# PATIENTS / CYCLES	37/42	34/34
AVG. AGE \pm S.D.	32.6 \pm 3.70	33.6 \pm 3.51
AVG. # OOCYTES RETRIEVED	31.6	-
AVG. # MII OOCYTES \pm S.D.	22.8 \pm 10.9	-
AVG. # MII VITRIFIED \pm S.D.	13.1 \pm 8.9	-
AVG. # MII WARMED \pm S.D.		9.6 \pm 7.48
SURVIVAL RATE \pm S.D. (n)	-	82.9%
AVG. No. OOCYTES ICSI \pm S.D.	9.2 \pm 5.31	8.0 \pm 6.72
FERTILIZATION RATE \pmS.D. (n)	71.5%	77.9%
BLASTOCYST RATE \pmS.D. (n)	43.0%	49.8%
AVG. # EMBRYOS TRANSFERRED	1.4	1.7
IMPLANTATION RATE (n)	16%	25%
CLINICAL PREGNANCY RATE (n)	33%	41%

Fertility Preservation



**Million
Dollar
Question!**

How many eggs to cryopreserve?

RBA experience: IVF patients 30–39 years

Prospective controlled study to evaluate laboratory and clinical outcomes of oocyte vitrification obtained in in vitro fertilization patients aged 30 to 39 years. Chang CC, Elliott TA, Wright G, Shapiro DB, Toledo AA, Nagy ZP. FS 2013

	Young 30–36 y (n=11)	Advanced 37–39 y (n=11)	P
Patient age (mean±SD)	32.9±1.9	37.9±0.8	<.01
Survival rate (%)	82.5	76.4	NS
Fertilization rate (%)	70.1	62.9	NS
Day 3 good Embryo (%)	55.6	40.4	<.05
Embryos transferred	24 (2.18)	29 (2.64)	NS
Clinical pregnancies (%)	7/11 (63.6)	3/11 (27.3)	NS
Implantations (%)	10/24 (41.7)	6/29 (20.7)	NS
Take home babies (%)	6/11 (54.5)	2/11 (18.2)	NS
No. of live births	8	3	–
Oocyte to Live birth (%)	8/97 (8.2)	3/89 (3.3)	NS

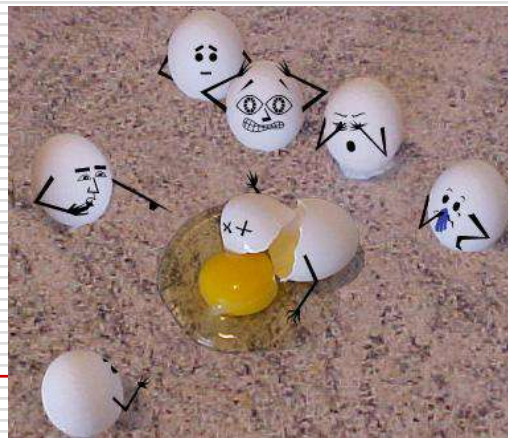
Chang et al FS, 2013

NEED 12 EGGS

NEED 30 EGGS

Safety

Oocyte cryopreservation



Live Birth Data from Egg Cryo from RBA

Including deliveries until the end of 2011

	Fresh Donor	Cryo Donor
No. of patients / Deliveries	58	257
Recipient Age	39.9 ±5.6	41.3 ±4.5
Live births (infants born)	91	338
Term delivery 37 weeks	28	188
Congenital anomaly*	3 / 58	5 / 257
All deliveries	2659.4 ±690.9	2938.3 ± 770.0
Singleton/twin/triplet deliveries	26/31/1	178 / 77 / 2
Term deliveries	3361.2 ±677.2	3518.8 ± 585.2

Down sy. 2xHemangioma

Congenital anomalies: heart murmur, 1 baby died at 2 months with multiple complications, cleft lip/palate, club foot, spina bifida (TAB)

Next step:

From “Traditional” ➔ to Automation



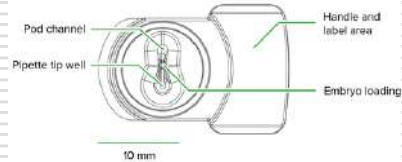




Automated vitrification



- Semi-Automated the equilibration process
 - Consistent timing
 - Volume
 - Temperature
 - No scientist variation
- Standardized process
Closed system



Automated vitrification - RESULTS

- Embryo Randomization
- Patients must have at least one embryo suitable for TE biopsy and freezing on day 5 or day 6
- Embryo assignment was based on Patient Number and blastocyst grading criteria

Embryos suitable for biopsy and freeze	ODD patient number #	EVEN patient number #
1 st embryo	Gavi	Cryotop
2 nd embryo	Cryotop	Gavi
3 rd and/or additional embryos not included in this study		

Automated vitrification

Clinical Results: Recovery and Survival Rates

Values	Gavi	Cryotop
Average of Age	36.21	36.24
# of Warmed	209	238
Recovered	207 (99.0%)	238 (100%)
Survived $\geq 75\%$	205 (99.0%)	235 (98.7%)
Embryo Transferred	205 (98.1%)	235 (98.7%)

Automated vitrification

Clinical Results: Outcomes to Fetal Heart

Values	Gavi	Cryotop
# of Embryo Transferred	205	235
Biochemical Pregnancy (+ β HCG)	137 (66.8%)	154 (65.5%)
Implantation Rate (FH rate)	119 (58.0%)	137 (58.3%)

Automated vitrification

Nace el primer bebé del mundo a partir de un óvulo vitrificado automatizadamente

15.11.2017 - 16:39h - EFE

- El bebé ha nacido en un hospital italiano tras un tratamiento en Barcelona.
- La vitrificación es un sistema de criopreservación ultrarrápido que reduce el tiempo y el riesgo de la formación de cristales de hielo.
- Hasta ahora, el proceso solo podía hacerse de forma manual.

Dispositivo cerrado herméticamente

La novedad que aporta este nuevo sistema, denominado GAVI, es que permite que la exposición del material biológico, en este caso los ovocitos, a los medios crioprotectores **se haga de forma automatizada** y en un dispositivo cerrado herméticamente evitando el contacto directo de los ovocitos con el nitrógeno líquido.

<http://m.20minutos.es/noticia/3187805/0/nace-primer-bebe-ovulo-vitrificado-automaticamente/>

Automated vitrification

A LOOK INSIDE SARAH



1. Sarah can vitrify up to 30 oocytes/embryos at once (in 6 Mini straws with 5 oocytes in each).
2. Sarah can vitrify up to 18 ovarian tissue slices (in 6 Maxi straws with 3 samples (10 X 5mm) in each).

Automated vitrification



- Operate with embryo tested CBS straws
- Manual preparation takes <5 min
- Vitrifies oocytes and embryos (safe open system)
- Cooling and warming rates of >20,000°C/min
- Can vitrify 30 oocytes simultaneously in 17 min.
- Automatic insertion into LN
- Semi-Automatic warming and dilution (Helia)

Automated vitrification

RESULTS AFTER VITRIFICATION OF MICE OOCYTES AND EMBRYOS USING SARAH

- 95% survival (19/20) with MII mice oocytes.
- 100% survival (20/20) with 8 cell stage embryos (80% (16/20) blastocyst rate).
- 100% survival (35/35) with blastocysts stage embryos (80% hatching).



Arav et al., 2016

Automated vitrification

HELIA - THE FIRST GLOBAL RAPID WARMING DEVICE FOR OPEN CARRIERS

- Allows fast warming rates $>300,000^{\circ}\text{C}/\text{min}$ and rapid movement into 22°C
- Fits all open carriers
- Can be operated manually or automatically
- Simple, fast and repeatable process
- Employs unique technology preventing from losing the sample



Conclusions

- Vitrification of oocytes (and embryos) provides excellent outcomes for both autologous and for donor cycles
- Significantly improved egg cryopreservation technology now provide options for patients in need for fertility preservation and for various other indications.
- Vitrification has changed our way of work.
- Automated vitrification has the potential to revolutionize egg/embryo vitrification; standardizing the process, increasing laboratory efficiencies.

