

Experience & The Practical Aspects of Donor Egg Banking

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Zsolt Peter Nagy, Ph.D., HCLD/CC(ABB)
Scientific Director
Reproductive Biology Associates
Atlanta, USA

Disclosure

Shareholder: MEB

Advisory Board Member: Origio, Unisense

Speaker Bureau: MERCK MSD; EMD Serono

Learning Objectives

- Recognize the various knowledge and skills needed to successfully cryopreserve oocytes.**
- Identify the various clinical and laboratory/technological aspects that is required to successfully manage donor egg cryo treatments.**
- Manage adequate patient handling (both donors and recipients) and system operation factors that impacts outcomes.**

Presentation review:

- History
- Needs/indications
- Safety issues
- Existing techniques
- Results of egg/embryo freezing
- Future Perspectives / Conclusions

Oocyte Freezing History: Human

1986: Slow freeze, DMSO (Chen, Australia)

1987: Slow freeze, DMSO (Van Uem, West Germany)

1989: Slow freeze, PROH and DMSO (Siebzehnrübl, West Germany)



1997: Slow freeze, PROH and Sucrose - ICSI (Porcu, Italy)

1998: Slow freeze, PROH and Sucrose - Immature/Donor oocytes
(Tucker, USA)

1999: Vitrification, EG and Sucrose - open pulled straws (Kuleshova,
Australia)

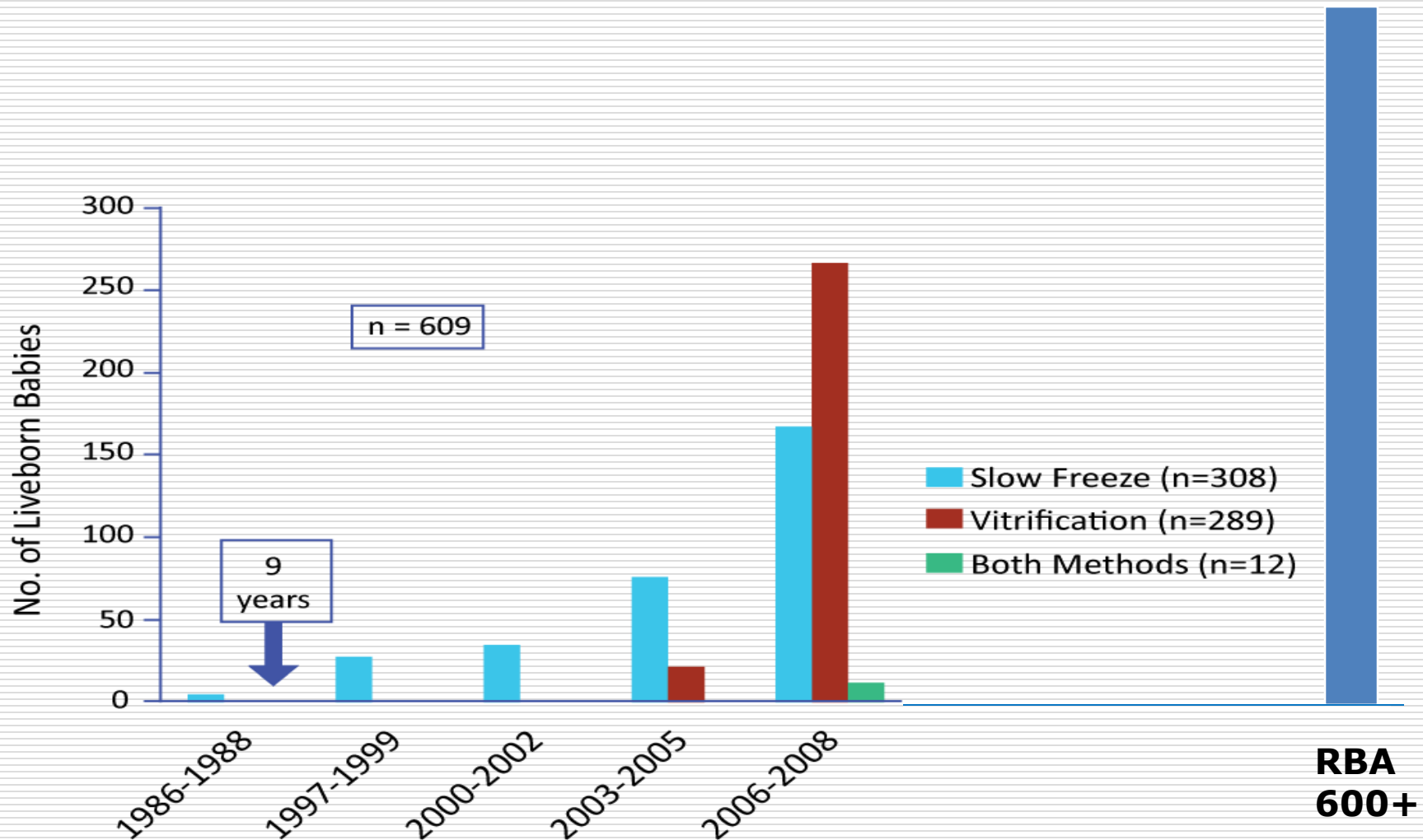
2000: Vitrification: EG and Sucrose - electron microscope grid (Yoon, Cha,
Korea)

2002: Slow freeze, Choline-based medium (Quintans, Argentina)

2003: Vitrification, EG, DMSO and Sucrose - Cryotop™ (Katayama, USA)

Oocyte cryopreservation

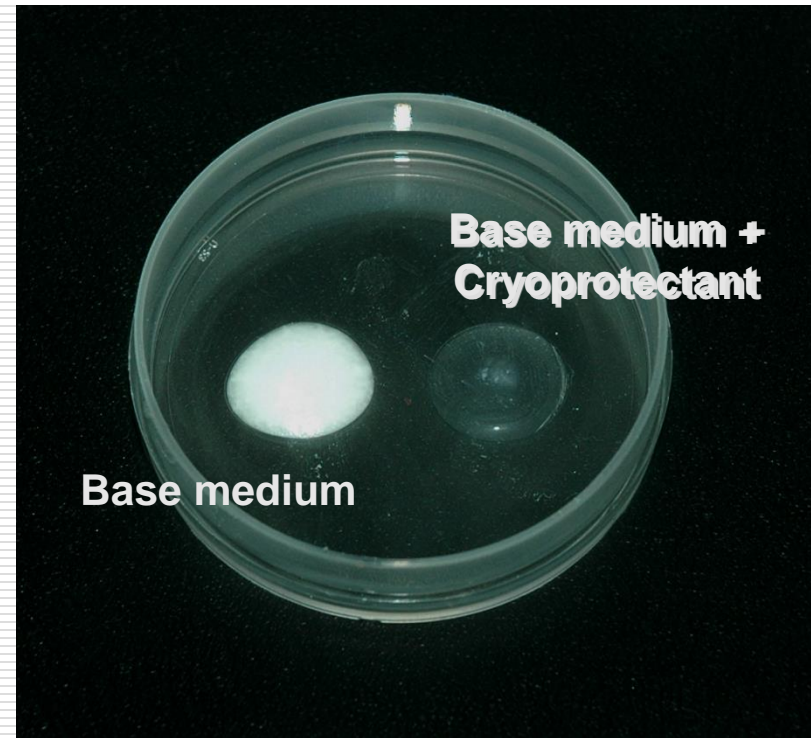
Number of Live Births 1986 to 2008



Why Vitrification?

Efficiency

Safety



Need for an efficient technique

Challenge: **Efficiency**

100-150 cryo eggs

1 pregnancy

(= 10-15 patients / trials)

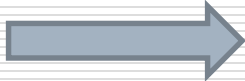


1986-2006
(slow freezing)

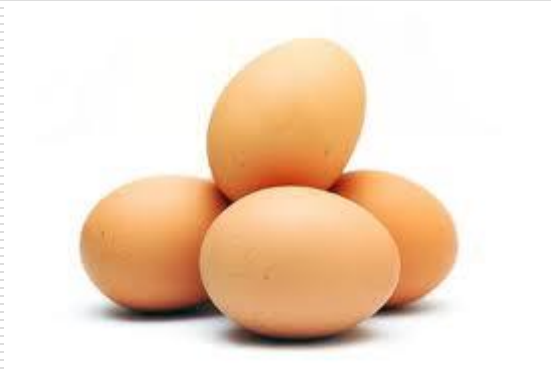


Need for an efficient technique

Challenge: **Solved today?**

4-5 cryo eggs  1 pregnancy

After 2007



Comparing Slow freezing with Vitrification

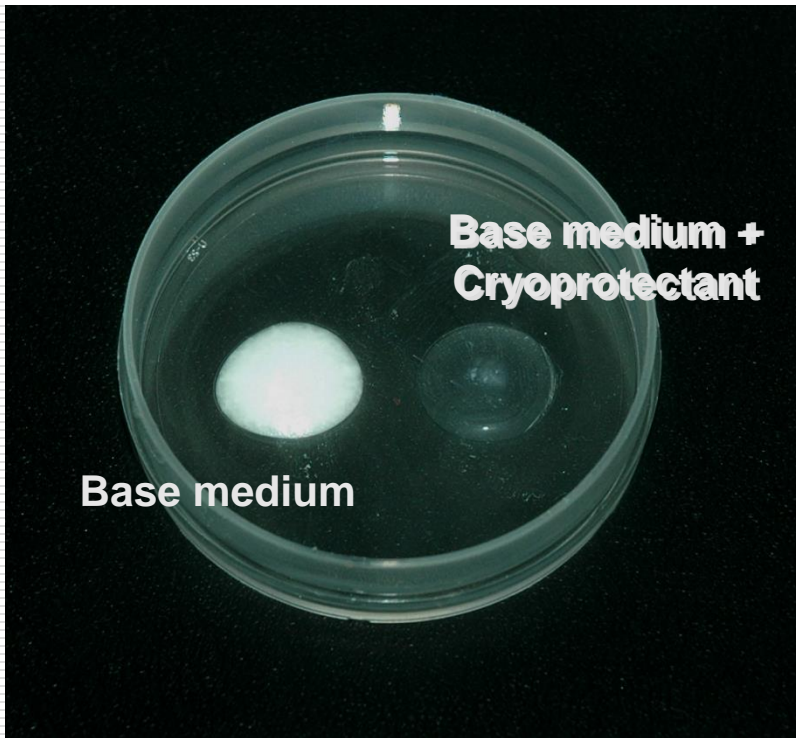
Slow freezing

Vitrification

Authors	Survival	Pregnancy	Authors	Survival	Pregnancy
Chen et al (1988)	75%	33%	Kuwayama et al (2005)	90%	41%
Li et al (2005)	90%	47%	Chian et al (2005)	94%	47%
Borini et al (2006)	74%	9%	Lucena et al (2006)	84%	57%
Barritt et al (2007)	86%	*75%	Cobo et al (2008)	97%	48%
Parmegiani et al (2008)	75%	19%	Nagy et al (2009)	88%	75%

WHAT IS VITRIFICATION?

Vitrification is a process that produces a glasslike solidification of living cells not by crystallization but by an extreme elevation of viscosity during the cooling



$$\frac{\text{COOLING RATE} \times \text{VISCOSITY}}{\text{VOLUME}}$$

Efficiency: Techniques

Slow Freezing

Vitrification

Before cooling



Physiological solution



Cryoprotectant solution



Vitrification solution



During cooling



Ice seeding

0.3°C/min

Slow cooling



Rapid cooling

Closed carrier OK



In LN2

Ultra rapid cooling

20,000°C/min

Open carrier



Efficiency: Techniques

Cryoprotective Agents

Permeating

Affect / pass through cell membranes

Interact with and replace H₂O

Lower freezing point

↑ Toxicity with ↑ T° and Concentration

PROH

DMSO

Glycerol

Ethylene Glycol



Increased

Permeability

Non-Permeating

Do not pass through cell membranes

Create osmotic gradient /
Dehydration

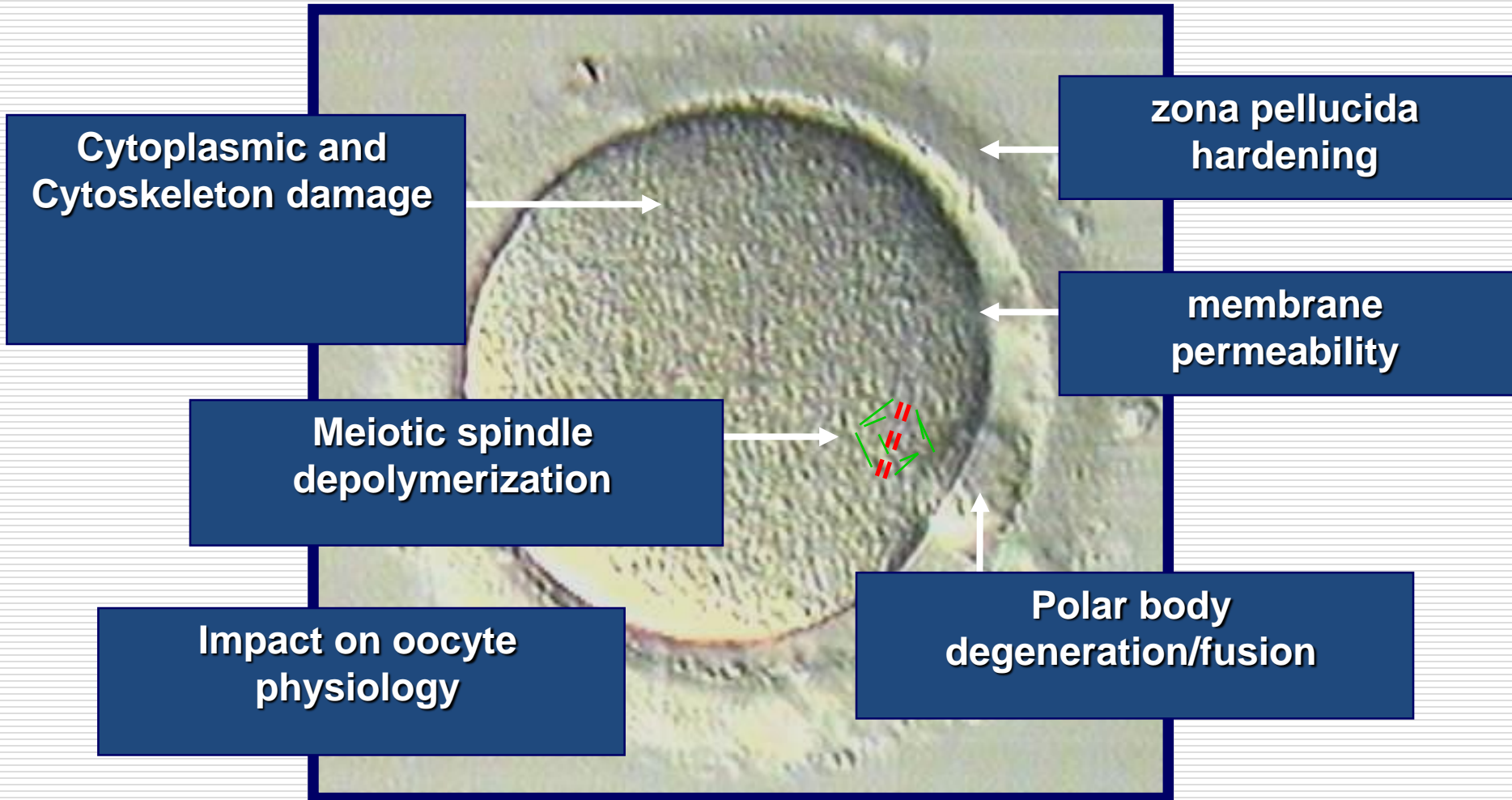
(High MW: >1000)

Glucose

Sucrose

Ficoll

Safety Issues



Indications for egg freezing

- **Government restrictions / legislation**
- **Fertility Preservation**
 - Medical
 - Social
- **Donor oocyte banking**
- **“Emergency” / Rescue**
- **Moral/Ethical/Religious**

Results

Autologous cycles

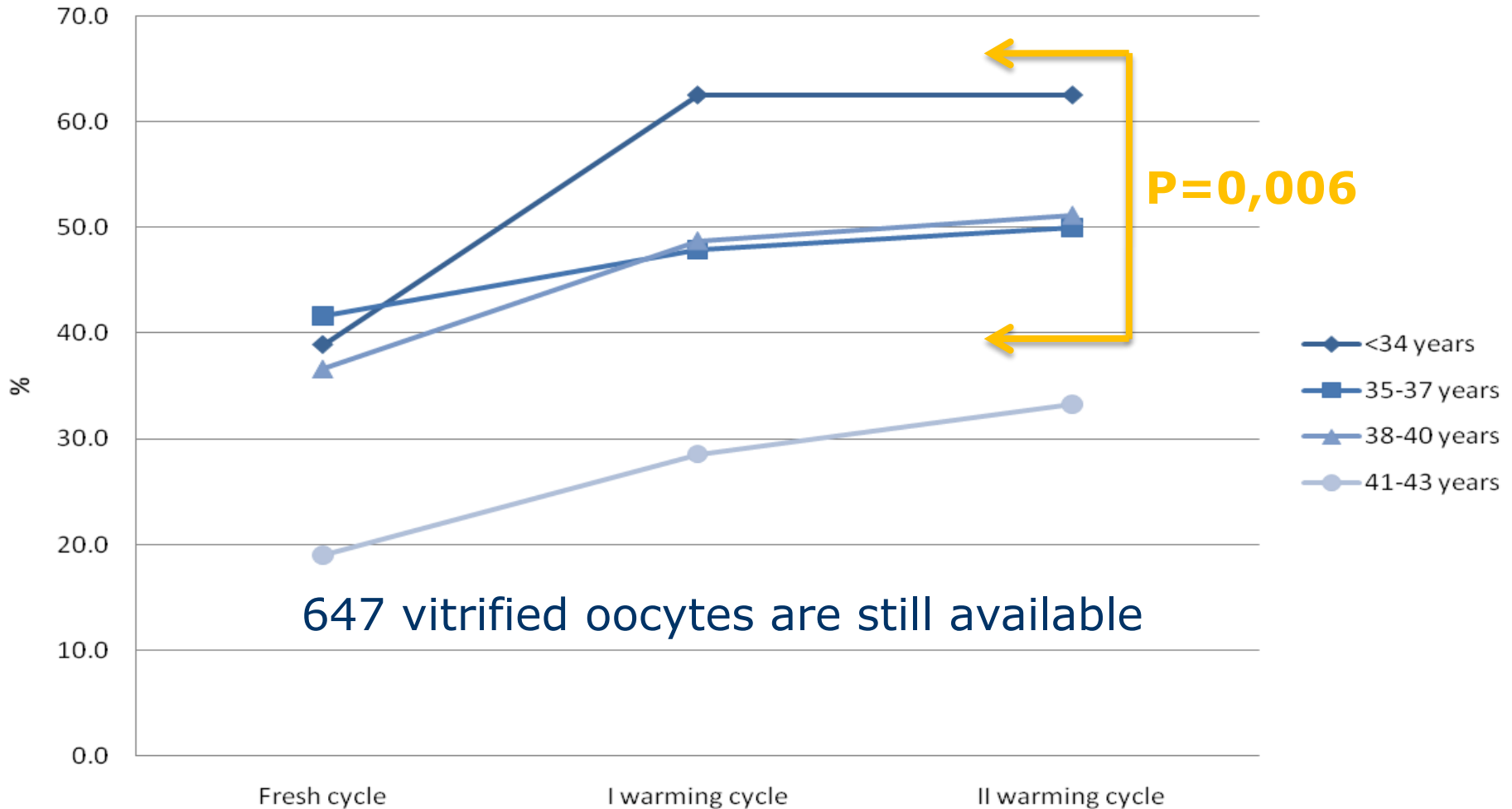
Efficiency: Results

Fertilization and embryo development of fresh and vitrified sibling oocytes

Rienzi 2010 HR	Fresh ICSI	Cryo ICSI (%)	<i>P</i>
Fertilization (2PN)	100/120 (83.3) ^b	95/124 (76.6) ^a	0.20
Normal 2PN morphology	96/100 (96.0) ^c	86/95 (90.5) ^c	0.16
Degenerated oocytes	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

Cumulative ongoing pregnancy rate achieved with oocyte vitrification and cleavage stage transfer without embryo selection in a standard infertility program.

Observed cumulative pregnancy rates according to female age

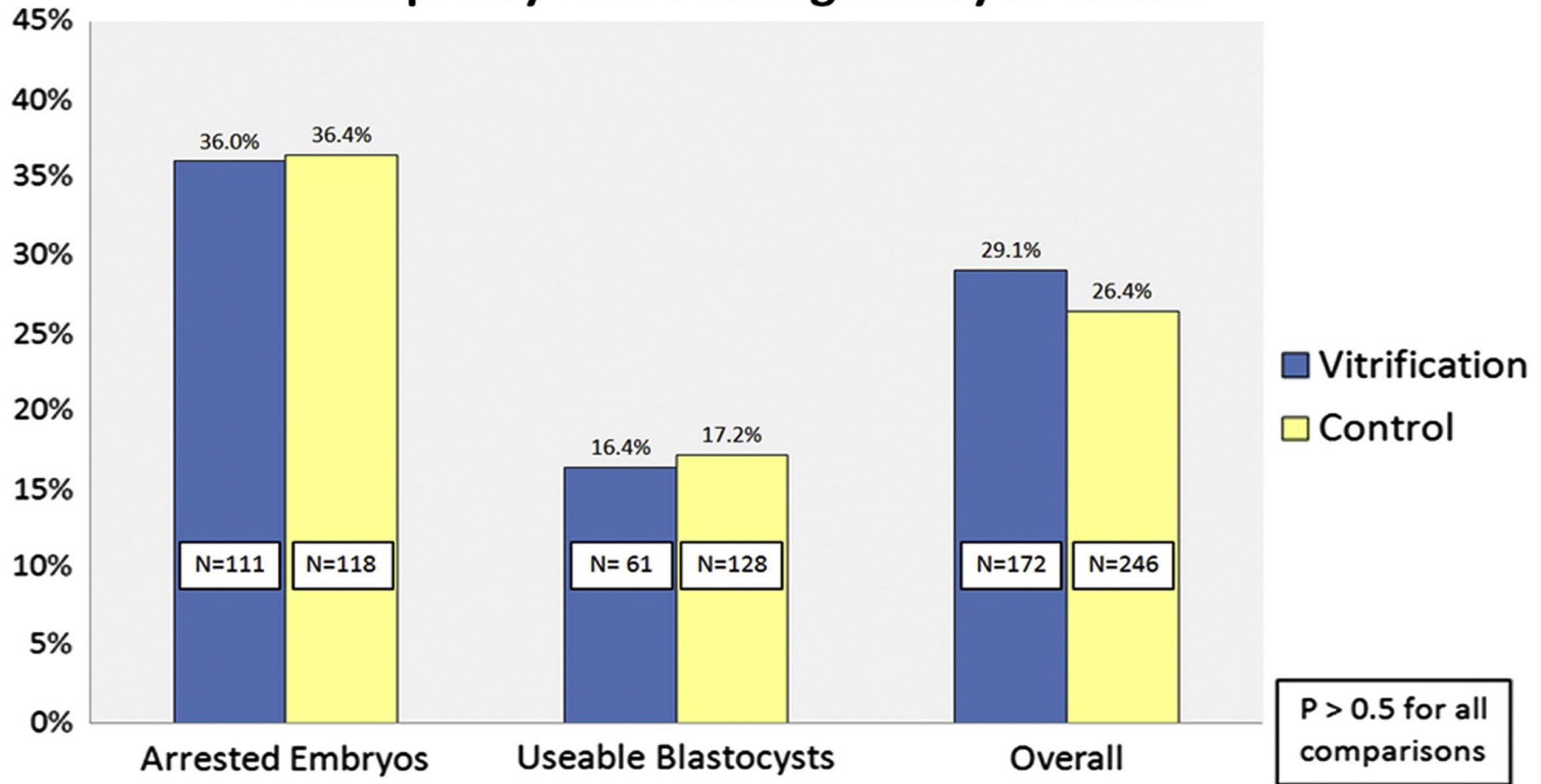


RBA experience: IVF patients 32–39 years

	Young 30–36 y (n=11)	Advanced 37–39 y (n=11)	<i>P</i>
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

Oocyte vitrification and embryonic aneuploidy

Aneuploidy Rate Among Embryos Tested



Forman et al.

FS 2012

Forty-four patients with a mean age of 29.9 ± 2.3 , 588 eggs

Results

Donor Egg Banking

RBA experience on oocyte vitrification

	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

Disadvantages of “fresh” ovum donation programs

- **Complexity in synchronization between donor and recipient**
- **Long waiting lists**
- **Uncertainty regarding the date of the donation**
- **Long E2 replacement**
- **Limited choice**
- **No quarantine period (HIV and others infectious agents)**
- **Supernumerary embryos cryostorage (moral ?)**

+ Oocyte-banking alleviates logistic aspects

6 years-experience of an ovum donation program using cryo-banked oocytes.

RBA experience on oocyte freezing: cryo egg bank (donor)

Donor selection:	young (<31; mean 27y.) & healthy
Stimulation:	rFSH with antagonist or agonist
Egg collection:	36 h post hCG and decumulation
Vitrification sol.:	Ethylene glycol & DMSO
Warming:	Three steps; 1.0 M, 0.5 M, 0 M sucrose
ICSI:	3 h post thaw / ET on Day 5
Recipient:	Usual LEP, 6 eggs per warming

RBA experience on oocyte freezing Cryo Egg Bank (donor)

342 Don. 463 cl. (26.5y.) 11553 Vit (24.9/don)

1145 Rec. 41.1y

7063 Warmed (6.2/R.)

Survived

6338 (89.7%)

Fertilized

5348 (84.4%)

Blastocysts

3497 (65.4%)

No of Es for ET

1579 (1.38 / Recip.)

No of Es for Cryo

1928 (1.68 / Recip.)

RBA experience on oocyte freezing Cryo Egg Bank (donor)

ET **1145 Transfers**

+FCA **652 (56.9%)**

No of FCAs **676 (42.8%)**

HCG versus Lupron trigger

Nagy et al.,RBA 2010	Antagonist	Antagonist	P value
	+ HCG trigger	+ Lupron trigger	
# of donor (mean age±SD)	93 (26.35±2.9)	9 (26.57±2.54)	P=0.8265
# of recipient (mean age±SD)	207 (41.05±4.75)	19 (39.47±4.04)	P=0.1619
# of egg warmed (mean±SD)	1325 (6.40±1.99)	108 (5.68±0.94)	P=0.1205
# of egg survived (%)*	1150 (86.8%)	103 (95.3%)	P=0.0064
# of egg fertilized (%)	999 (86.8%)	93 (90.3%)	P=0.3604
# of embryo cleaved (%)	976 (97.7%)	92 (98.9%)	P=0.7144
# of ET (mean±SD)	419 (2.02±0.43)	35 (1.84±0.37)	P=0.0790
# of (+) hCG (%)	149 (71.9%)	13 (68.4%)	P=0.7916
# of Clinical PR(%)	126 (60.8%)	11 (57.9%)	P=0.8102
# of Implantation (%)	193 (46.0%)	12 (34.3%)	P=0.2168

Fresh vs. vitrified donor egg outcomes

(same donors May 2006- March 2009)

	Cryo oocyte	Fresh oocyte	P
Number of donors	81	81	NA
Number of recipients	100	91	NA
Mean age (\pmSD) of recipients	40.9 (\pm4.9)	41.2 (\pm4.7)	NS
Mean number of oocytes per recipients	7.1	25.28	<.001
Mean number of oocytes for ICSI	6.0	15.0	<.001
Average 2PN ICSI fertilization rate	77%	57%	<.001
Implantation Rate	52%	56%	NS
Mean number of embryos cryopreserved	1.5 (\pm1.5)	12.5 (\pm8.8)	<.001
Clinical pregnancy rate	67%	69%	NS
Multiple Pregnancy rate	44%	46%	NS

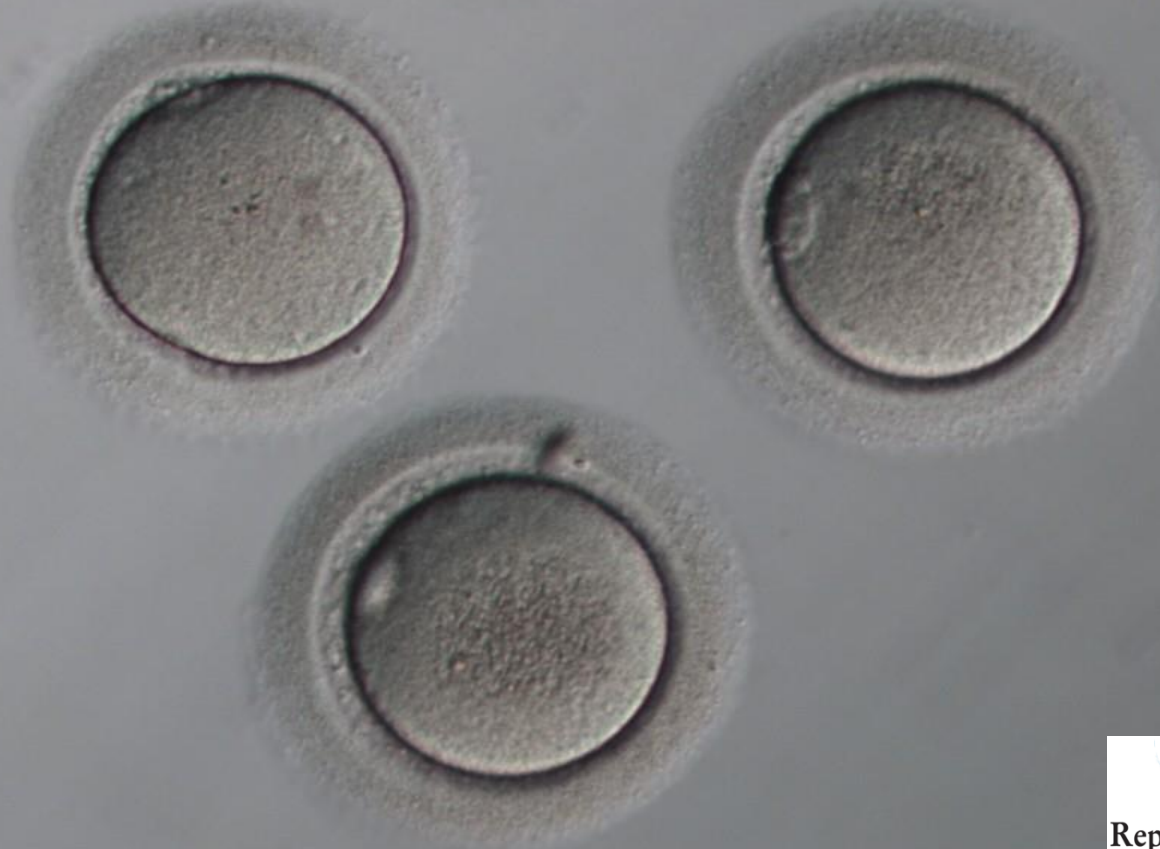
Egg-banking in ovum donation. RCT

	Egg- bank	Fresh	P value
Number of subjects	295	289	
MII oocytes retrieved	3286 (11.1 ±3.2)	3185 (11.0 ±2.8)	0.634
Survival rate	3039 (92.5)	-	-
Oocytes inseminated	3039 (10.3±2.9)	3185 (11.2 ±3.4)	0.091
Fertilization rate (2PN)	2256 (74.2)	2334 (73.3)	0.393
Top quality day-3 embryos/inseminated oocyte	1098 (36.1)	1201 (37.7)	0.198
Clinical Pregnancy Rate	50.2%	49.8%	NS

Consistent results in two unrelated Egg Banks

	IVI	RBA
Number of donation cycles	1051	168
Number of recipient cycles	919	322
Mean age (\pm SD) of recipients	41.2 \pm 4.3	41.1 \pm 4.9
Total (Mean \pm SD) number of oocytes warmed per recipient	12786 (12.9 \pm 4.0)	2001 (6.2 \pm 1.9)
Total (Mean \pm SD) number of oocytes for ICSI	11949 (11.4 \pm 3.4)	1750 (5.4 \pm 1.7)
Average 2PN ICSI fertilization rate	8920 (74.7)	1494 (85.4)
% of Good quality Embryos on Day-3 (per inseminated oocyte)* ¹	5366/11949 (44.9)	979/1750 (55.9)
% of Good quality Embryos on Day-5 (per embryo subjected to extended culture)* ¹	1427/3568 (39.9)	582/1185 (49.1)
Implantation Rate	655/1655 (39.6)	255/577 (44.2)
Total (Mean \pm SD) number of embryos cryod	1915 (1.8 \pm 2.0)	414 (1.3 \pm 1.5)
Clinical pregnancies (rate /transfer) * ²	502 (55.4)	182 (56.5)
Infants born* ³	343 (180 female;163 male)	146 (64 female;82 male)

Is elective single embryo transfer a viable option in oocyte cryopreservation program?



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...



Frozen Embryos From Frozen Eggs

100 patients (Cryo Egg Bank) Cryo Embryo

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

Practical questions

Transporting vitrified oocytes (embryos)?

	Control	Nitrogen vapor shipment	<i>P value</i>
# of Recipient	6		NA
# of donor	6		NA
# of egg warmed	21	12	NA
# of egg survived	19/21 (90.4)	12/12 (100)	0.5227
# of fertilized (%)	12/21 (57.1)	11/12 (91.6)	0.0545
# of grade A embryo on Day 3 (%)	9/21 (42.8)	6/12 (50.0)	0.7307

Experience of two Egg Banks

My Egg Bank-North America (MEB-NA)

Donor Egg Bank USA (DEB USA)

My Egg Bank-NA

- Established in 12/2010
 - Core partnerships
 - Seattle Reproductive Medicine
 - Reproductive Science Center of New England
 - Center for Reproductive Medicine-Orlando
 - Reproductive Biology Associates-Atlanta
 - Currently inventory is held by partner centers
 - Agreements with Affiliated clinics
 - 60 + affiliated practices
 - Tech
 - Non-tech

My Egg Bank-NA

Frozen Donor Egg cycles; 2006-2013*

- Approximately 2000 completed cycles
- Approximately 1150 delivered or ongoing pregnancies
- Approximately 1000 babies born (includes twins)
- MEB partners with 1.4- 1.7 embryos per ET (2013)
- Anticipated demand for 2014
 - 1200 cycles
 - 10,000 vitrified oocytes needed
 - 400+ donors required to fill demand

**Prior to October, 2012 all MEB cycles were performed at RBA or with oocytes vitrified in the RBA lab. After October, 2012 cases were performed network-wide with eggs from all four centers.*

First full year of operation as a network-2013

- **Egg “Production”**
 - 337 donor retrievals
 - 7881 eggs vitrified
 - 6-8 eggs/lot; approx 1200 egg lots generated
- **Egg Shipping**
 - 672 egg lots shipped
- **Training**
 - 30 + Centers trained at RBA
- **Utilization**
 - 628 embryo transfers at the four partner centers,
 - Approx 200 ET in the affiliate centers

2013 Preliminary Outcomes

- **Partner Centers**

- **4600+ eggs warmed**

- **87% survival***

- **88% fertilization***

** Estimates based on incomplete data*

- **613 transfers**

- **421 positive hCG (69%)**

- **Approx 360 ongoing (59%)**

- **More than 60% done as SET**

- **Over 50% of cycles with additional embryos for cryo***

Affiliate Outcomes

- **Affiliates are obligated to report embryology and clinical outcomes**
- **Contracted to maintain embryology and clinical benchmarks**
- **Remediation required if benchmarks are not met**

Top performing MEB affiliate #1

- **24 Egg Thaws**
 - Survival > 90%,
 - Fert Rate >85%
 - 23 ET, 22 SET, 1 DET
 - 18/24 + Beta hCG (75%)
 - 17/24 ongoing(70.8%)
 - 75% of patients had embryos cryo'd

- *Eggs sourced at all four partner centers*

Top performing MEB affiliate #2

- **49 Egg thaws**
 - **>90% survival**
 - **>85% fertilization**
 - **46 ET, 2 'freeze all', 1 No ET**
 - **Average # of embryos transferred = 1.37**
 - **39/49 + Beta hCG (75.5%)**
 - **31/49 ongoing (63%)**
 - **47% of cases with embryos for cryo**

Example of an MEB center with multiple areas for concern

- 7 egg thaws thus far
 - 46 eggs warmed
 - 43 survived (93%)
 - 38 X 2PN (88%)
 - 12 'good quality embryos' on d3 (31%)
 - 10 blasts (26%)
 - 1 ongoing pregnancy
 - Day 3 ET X 2 both negative
 - Day 5 DET X 5 one positive

MEB affiliate case study

■ Prior to remediation

- 18 egg thaw cycles
- Good survival, fertilization, and adequate blastulation rate (48%)
- Only 4 ongoing pregnancies (22%)
- *Review requested by MEB after 11th egg thaw (3 + beta hCG)*
- *Recipient Protocol deviations noted in review of cycle summaries*
- *Center advised to follow MEB approved replacement protocols*

■ After remediation

- 12 egg thaw cycles
- Equally good embryology
- 9 ongoing pregnancies (75%)

Operational overview

- Central office records each registrant to the Egg Bank
- Central office maintains web based donor roster and inventory adjustments
- Centralized staff monitors protocol across the network and collects outcome data
- Central staff generates Standards for Operation and monitors adherence
- Regular teleconferences for laboratory, clinical, business and nursing directors

Operational overview

- Network-wide standards for
 - Donor screening/selection
 - Genetics/history/health/FDA
 - Psych Evaluation
 - AMH/BAF
 - Donor management
 - Stim protocol
 - Agonist trigger
 - Recipient screening
 - Male factor minimums
 - Uterine factor
 - Replacement Protocol
 - Lab standards

Clinical Elements of Success

- Careful donor selection
- Adherence to consistent standards for donor screening and management
- Strict adherence to recipient screening paradigms
- Standardized recipient replacement protocols
- Centralized management to reduce the number of ‘cooks’
- Minimalist approach
- Case review

DEB USA Multi-Site Experience

2012 – December 26, 2013

# thaw cycles	626
Average #/thaw	6.2
# eggs thawed	3,881
# survived	3,337 (86%)
# 2PN	2,536 (76%)
# transfers	582
# cancelled	44 (7%)

DEB USA Multi-Site Experience

2012 – December 26, 2013

All Transfers Day 3 and Blastocyst

Clinical Pregnancy/ET	291 (50%)
SAB	36
Ongoing Pregnancy	255
Ongoing/ET	43.8%
Ongoing/Thaw	40.7%
Singleton	71%
Twin	28%
Triplet	< 1%

DEB USA Multi-Site Experience

2012 – December 26, 2013

Day 3 Transfers

# Day 3 ET	296
Average # ET day 3	1.8
Clinical Pregnancy/ET	119 (42%)
SAB	18
Ongoing/ET	101 (34%)

DEB USA Multi-Site Experience

2012 – December 26, 2013

Blastocyst Transfers

# Day 5 ET	286
Average # ET day 5	1.6
Clinical Pregnancy/ET	172 (60%)
SAB	18
Ongoing/ET	154 (54%)

Clinical Outcome by # Eggs Thawed

# of Eggs	# of Cycle	Preg	Clinical/ ET	Clinical/ Thaw	SAB	CX	Ongoing/ ET	Ongoing/ Thaw
9	2	1	50.0%	50.0%	0	0	50.0%	50.0%
8	17	5	31.3%	29.4%	0	1	31.3%	29.4%
7	176	85	51.2%	48.3%	12	8	44.0%	41.5%
6	341	154	47.7%	45.2%	23	18	42.1%	39.9%
5	101	46	54.8%	45.5%	1	17	53.6%	44.6%

DEB USA 2012-13

Frozen Egg Cycles

- **626 Thaw Cycles**
- **238 Cycles with Vitrified Blastocysts (1.8/cycle)**
- **428 Vitrified Blastocysts**
- **35% Vitrified Blastocysts expected to Deliver**
- **150 Babies**

Pregnancy Outcome per Number of Eggs Retrieved

#Eggs	# Transfers	Ongoing/ Thaw
>40	144	59 (41%)
30-39	179	85 (47%)
20-29	199	80 (40%)
15-19	95	40 (42%)
<15	26	12 (46%)
Total:	643	276 (43%)

#M2s	# Transfers	Ongoing/ Thaw
>40	10	4 (40%)
30-39	152	61 (40%)
20-29	203	97 (48%)
15-19	173	73 (42%)
<15	105	41 (39%)
Total:	643	276 (43%)

Internal Freeze – Internal Thaw

	Center 1	Center 2	Center 3 (SG)
n	15	12	57
Survival %	91.8%	86.1%	90.2%
Fert %	85.4%	73.3%	75.6%
Clev %	97.4%	93.2%	84.7%
Clinical Preg/ET	46.7%	40.0%	58.2%
Ongoing/Thaw	40.0%	25.0%	52.6%

Internal Freeze – External Thaw

	SG-MD
n	234
Survival %	89.9%
Fert %	76.7%
Clev %	95.9%
Clinical Preg/ET	49.6%
Ongoing/Thaw	41.9%
Ongoing/Egg Lot	48.5%

External Freeze – Internal Thaw

	SG-MD
n	142
Survival %	85.6%
Fert %	74.9%
Clev %	89.9%
Clinical Preg/ET	51.5%
Ongoing/Thaw	42.8%
Ongoing/Egg Lot	44.2%

Outcomes by Embryologist - Freezing

Freeze Tech	Dec n	Ongoing/ET
K-S	28	60.7%
PD-S	22	59.1%
JG-S	52	55.78%
JL-S	113	50.9%
TH-S	51	45.1%
K-R	22	40.9%
B-B	27	40.7%
TB-S	26	38.5%
S-F	26	26.9%
B-F	18	22.2%

Pregnancy Outcome Per Endometrial Thickness

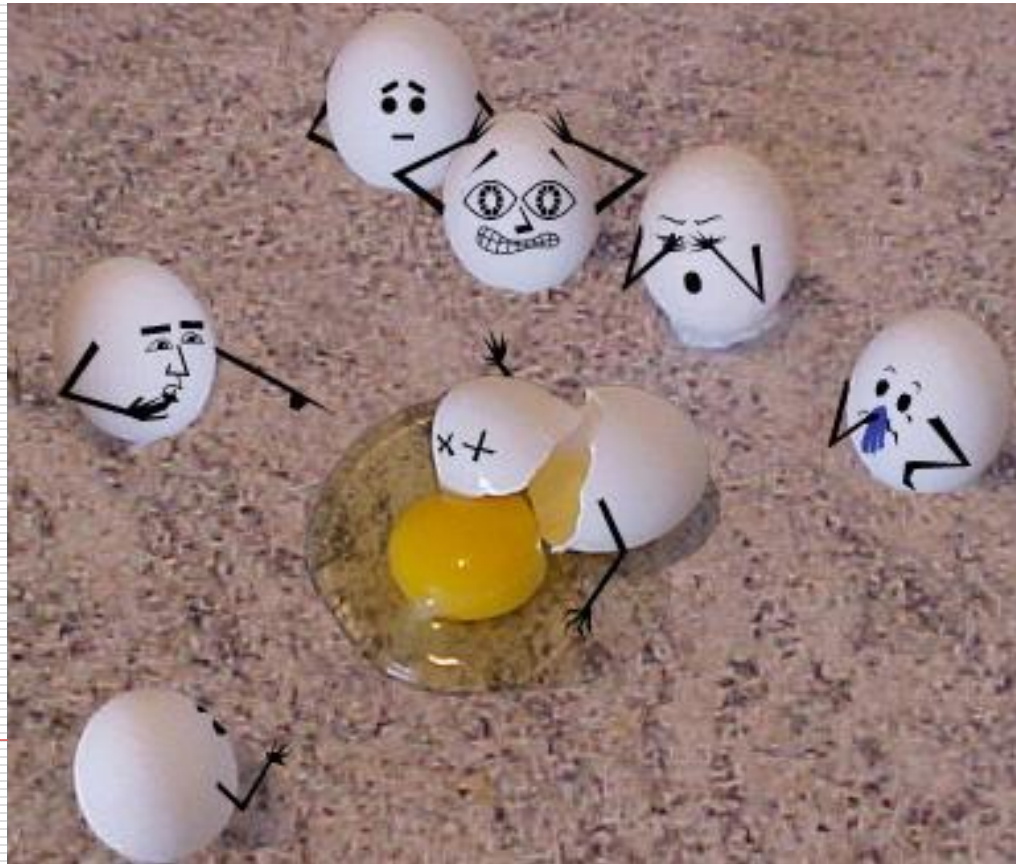
	<7.0mm	7-8mm	8-9mm	9-10mm	10-11mm	11-12mm	>12mm
Total ET	8	38	152	98	68	75	117
Clinical Pregnancy	2 (25%)	11 (29%)	70 (46%)	47 (48%)	39 (57%)	37 (49%)	56 (48%)
SAB	0	1	12	6	5	3	5
Ongoing	2 (25%)	10 (26%)	58 (38%)	41 (42%)	34 (50%)	34 (45%)	51 (44%)

What Causes a Poor Thaw Cycle?

- Donor
 - Stimulation
 - Inherent Oocyte Potential
 - Oocyte Response to Vitrification
 - Vitrification Technique
- Shipping/Transport
- Thaw
 - Technique
 - Sperm/Male Factor
 - Embryology Quality

Safety

Oocyte cryopreservation



Oocyte cryopreservation birth 'case reports' 1986–2008

Parameter	Cryopreservation method		
	Slow-freeze	Vitrification	Both
No. of embryo transfers	1974	834	19
No. of liveborn babies	282	285	12
Baby gender (gender information available for 168 slow-freeze, 189 vitrification and 12 both methods)	99 female, 69 male	86 female, 103 male	8 female, 4 male
Birth defects	1 ventricular septal defect, 1 choanal atresia, 1 Rubenstein-Taybi syndrome	2 ventricular septal defect, 1 biliary atresia, 1 clubfoot, 1 skin haemangioma	None

Adapted Noyes N, Porcu E Borini A. *Reprod BioMed Online* 2009.
<http://www.rbmonline.com/Article/3971> [e-pub ahead of print on 8 April 2009].

Live Birth Data from Egg Cryo from RBA

Updated by end 2011

	Fresh Donor	Cryo Donor
No. of patients / Deliveries	58	257
Recipient Age	39.9 \pm5.6	41.3 \pm 4.5
Live births (infants born)	91	338
Term delivery 37 weeks	28	188
Congenital anomaly*	3	5
All deliveries	2659.4 \pm690.9	2938.3 \pm 770.0
Singleton/twin/triplet deliveries	26/31/1	178 / 77 / 2
Term deliveries	3361.2 \pm677.2	3518.8 \pm 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)

Conclusions



Oocyte Vitrification:

- Same fertilization, embryo development
- Similar implantation / pregnancy rates

Outcomes does not raise concern so far
on safety of oocyte (embryo) vitrification
(Registry would be useful, SART, ASRM)

Conclusions



Donor Egg Banking provides proven benefits:

- **No need for synchronization**
- **No waiting – start at any time**
- **Large donor selection – easy match**
- **Quarantine is possible**
- **Results similar to fresh egg donation**
- **Few supernumerary embryos (less ethical concerns)**
- **Economically less burdensome**

Acknowledgment

EMBRYOLOGISTS

Jeremy Chang PhD

Graham Wright, BSc

Stacey Jones, BSc

Diana Patricia Bernal, DVM

Ann Fisher, BSc, MPH

Wendy Brockman, BSc

Thomas Elliott, BSc

PHYSICIANS

Hilton Kort, MD

Carlene Elsner, MD

Dorothy Mitchell-Leef, MD

Andrew Toledo, MD

Scott Slayden, MD

Robert Straub, MD

Daniel Shapiro, MD