2 PN and embryo cryopreservation is a well established procedure since many years.

Oocyte cryopreservation is a more recent procedure (still experimental ?)

Freezing and thawing can produce cellular demage at any stage of development

Oocyte cryopreservation is an alternative to zygote and embryo freezing

- When a sperm sample is not available
- In those countries where the cryostorage of embryos is forbidden by law
- Ethical concerns
- When women has to undergo chemo/radio-therapy treatments

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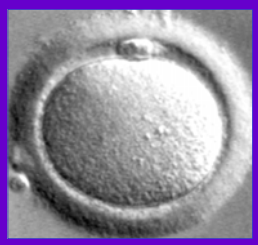
Embryo cryopreservation is forbidden in :

- Bangladesh
- El Salvador
- Germany
- Switzerland
- Italy

2 PN is not an embryo in Germany and Switzerland and can be cryopreserved



…. while is considered an embryo in Italy

Oocyte is the only stage available for cryopreservation

Since the legislation on ART passed in Italy in March 2004 limiting to 3 the maximum number of eggs to be inseminated and banning embryo freezing, **cryopreservation of spare oocytes entered the clinical practice.**

After 5 years :
oocyte cryopreservation can work !

Efficacy – Efficiency – Safety ?

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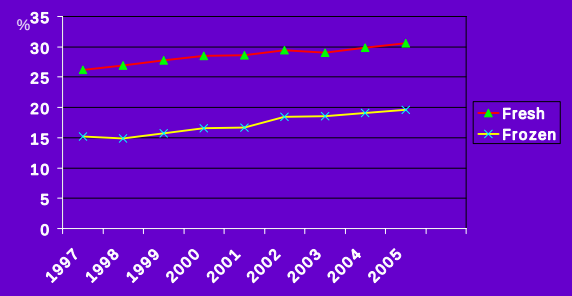
Italian Register
Oocytes cryopreservation

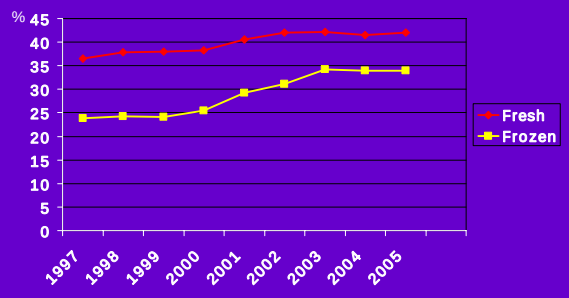
Year	2004	2005	2006	2007	Tot.
Frozen oocytes	14.234	25.489	28.784	27.513	96.020
Thawed cycle	783	2.711	2.977	2.994	9.465
Thawed oocytes	3.600	12.689	15.338	14.890	46.517
Survival rate	40%	45%	49%	50%	47.5%
Trasferred cycles	640	2.261	2.366	2.428	7.695
Pregnancies	41	257	298	327	923
PR/ET	6.5%	11.4%	12.6%	13.5%	12%

Italian data collection
Fresh vs Frozen PR/ET
(Oocyte cryopreservation)

Europe Data collection
Fresh vs Frozen PR/ET
(Embryo cryopreservation)



USA data collection
Fresh vs Frozen PR/ET
(Embryo cryopreservation)



Oocyte cryopreservation
S.I.S.Me.R. experience

	Before the Law	After the Law
Indications	- No semen available - Patient's ethical concern for embryo freezing - Some cases in ED programme	- All cycles with > 3 eggs - Elective cryo for OHSS risk
Cycles	93	657

Oocyte cryopreservation before the Law (1997-2003)

VITRIFICATION

N° cycles with vitrified oocytes	93
N° vitrified oocytes	508
N° cycles with thawed oocytes	42
N° thawed oocytes	207
N° intact oocytes (%)	115 (56)
N° fertilized oocytes after ICSI (%)	69 (60)
N° embryos (%)	46 (67)
N° transferred embryos	26
N° transferred cycles (%)	17 (40)
N° clinical pregnancies (%)	3 (18)
Implantation rate per embryo (%)	11.5
N° of deliveries	1

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Human Reproduction, Vol. 14, No. 12, 3077-3079, December 1999

Birth following vitrification of a small number of human oocytes: Case Report

Lilia Kuleshova[1], Luca Gianaroli[2], Cristina Magli[2], Anna Ferraretti[2] and Alan Trounson[1,3]

[1] Centre for Early Human Development, Monash Institute of Reproduction and development, Monash University, Wright Street, Clayton, Victoria, Australia and [2] SISMER srl , Bologna, Italy

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OOCYTE VITRIFICATION PROTOCOL

AT 37°C:

10% (v/v) ethylene glycole in PBS + 10% HSA	40"
20% (v/v) ethylene glycole in PBS + 10% HSA	30"
40% (v/v) ethylene glycole, 20.54% (w/v) sucrose in PBS + 10% HSA	60"

↓

Open Pulled Straw

oocyte ↓ air

LN_2

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OOCYTE THAWING PROTOCOL

AT 37°C:

0.4 M sucrose in PBS + 10% HSA	2 min
0.25 M sucrose in PBS + 10% HSA	2 min
0.125 M sucrose in PBS + 10% HSA	2- 3 min
PBS + 10% HSA	2- 3 min

↓

culture

↓

3 – 4 hours

↓

ICSI

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Oocyte cryopreservation
S.I.S.Me.R. experience

	After the Law
Indications	- All cycles with > 3 eggs - Elective cryo for OHSS risk

MATERIAL AND METHODS

Slow freezing protocol

2 step procedure:

- 1.5 M PrOH
- 1.5 M PrOH in 0.3 M sucrose

Thawing

Solutions with a decreasing concentration of cryoprotectant:

- 1M PrOH + 0.3M sucrose
- 0.5M PrOH + 0.3M sucrose
- 0.3M sucrose

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Cumulative Results 2004-2008

Indication 1 : surplus oocytes

N.of thawing cycles	657
N.of thawed oocytes	3273
N.of survived oocytes	2281 (70%)
Fertilization rate(%)	74%
N.of transferred cycles	549(82%)
N. of pregnancies(PR/ET)	85 (15.5%)
Implantation rate	10%
N. of abortions (%)	23 (27,%)
Ongoing pregnancy rate	11.3%

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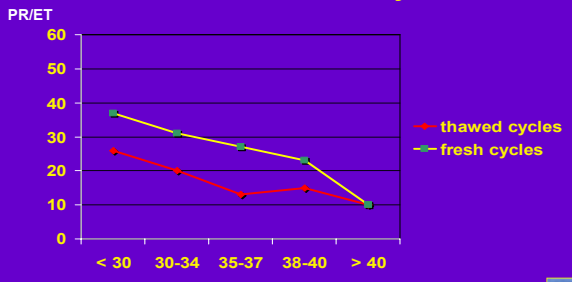
Results according to age

Fresh vs Frozen oocytes



Cumulative data : PR/ET fresh vs frozen : p<0.05

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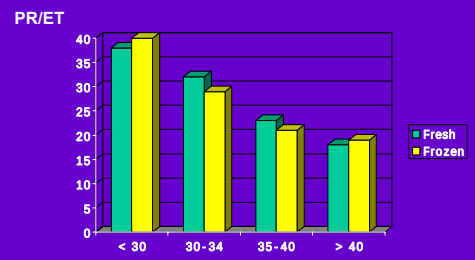
Indication 2 : Elective oocytes cryopreservation for OHSS risk (248 cycles)

Elective oocytes cryopreservation for OHSS risk



No statistical differences

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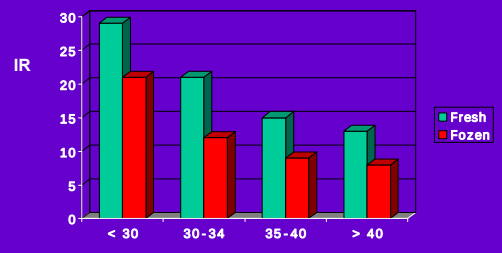
Implantation rate
Fresh vs frozen oocytes



P< 0.05

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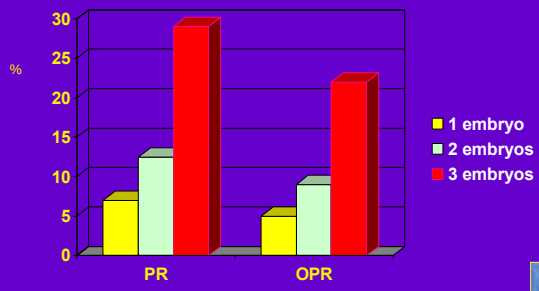
Results according to
the n°of embryos transferred



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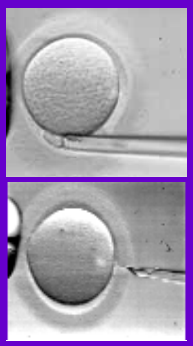
OOCYTE SELECTION BASED ON CHROMOSOMAL ANALYSIS OF PB1



One hour after collection, oocytes are denuded and PB biopsy on MII starts immediately

Up to 3 euploid oocytes are selected for ICSI

At 16 hours after insemination, oocytes were checked for the presence and morphology of pronuclei, nucleoli and polar body

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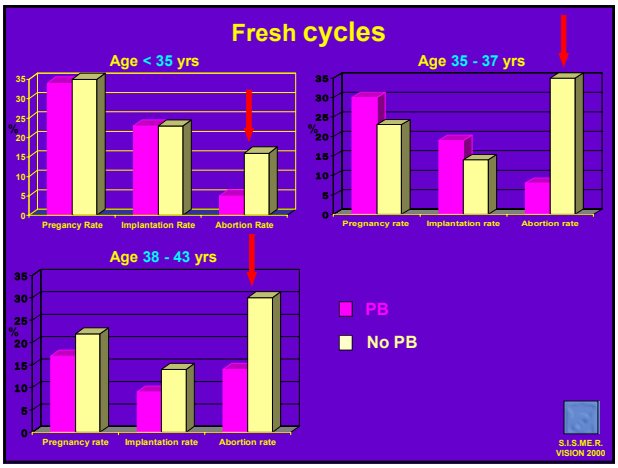
Fresh cycles
Age < 35 yrs
Pregancy Rate
Implantation Rate
Abortion Rate
Age 35 - 37 yrs
Pregnancy rate
Implantation rate
Abortion rate
Age 38 - 43 yrs
Pregnancy rate
Implantation rate
Abortion rate
PB
No PB
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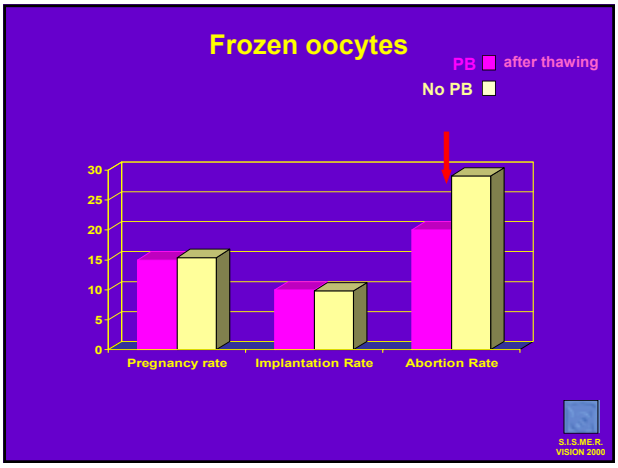
Frozen oocytes
PB after thawing
No PB
Pregnancy rate
Implantation Rate
Abortion Rate
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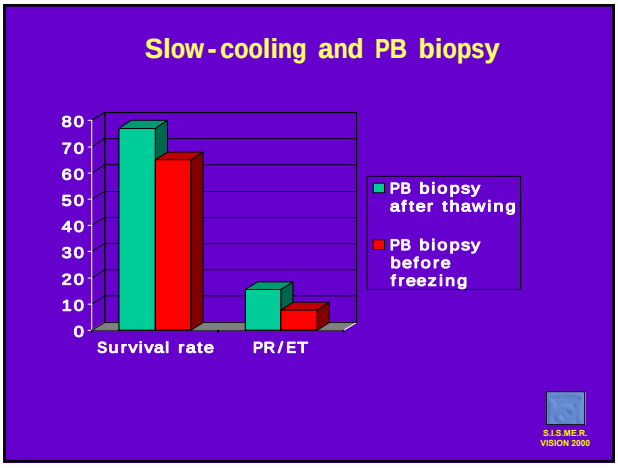
Slow-cooling and PB biopsy
PB biopsy after thawing
PB biopsy before freezing
Survival rate
PR/ET
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Slow- cooling oocyte cryopreservation

At present, oocyte cryopreservation is beneficial only in optimal conditions: young patients, high quality oocytes surviving the procedure.

To reach significantly better results, three embryos need to be transferred, exposing the patients to the risk of multiple pregnancies.

Cryopreservation affects viability of biopsied oocytes

New techniques, as vitrification , are under evaluation to increase oocyte freezing efficiency

Factors influencing the success of cryopreservation

1. Possible temperature shocks (+ 15°C or -5°C)
2. Possible changes in the plasma membrane
3. Selection of the right cryoprotectant
4. Dehydration: intensity and time
5. Critical cell volume
6. Solute concentration
7. Cooling rate
8. Thawing rate

The most important principle of the cryopreservation of the oocytes and embryos is:

The formation of ice crystals which should be avoided during the process of freezing of the cells and tissues

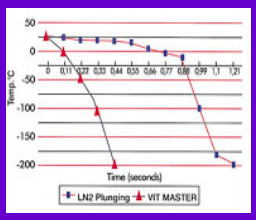
- The physical definition of vitrification is the solidification of a solution (water is rapidly cooled and formed into a glassy, vitrified state from the liquid phase) at low temperature, not by ice crystallization but by extreme elevation in viscosity during cooling.

Fahy 1984

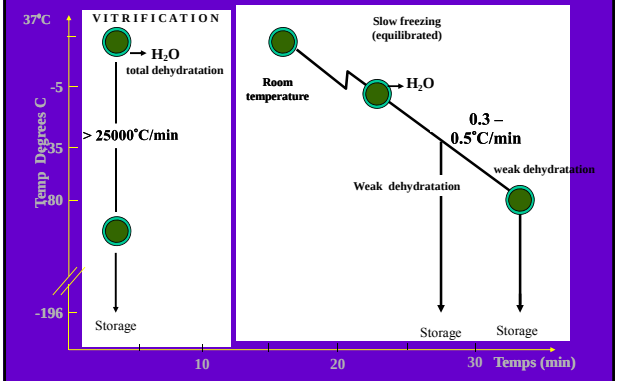
Freezing in the liquid nitrogen LN_2 (Vitrification)

Physical definition:

solidification of a solution to be similar to the state of the glass



Vitrification vs Slow cooling



Terminology

Instead of Freezing → Vitrification

Instead of Thawing → Warming

Historical review

- It was described at the end of the 18th Century
  (Tammann, 1898)
- Vitrification of mouse embryos at -196°C
  (Rall & Fahy, 1985; Ali and Shelton, 1993)
- Blastocyst development from bovine oocytes
  (Martino et al. 1996)
- Blastocyst development, Pregnancies, Deliveries from human vitrified oocytes, zygotes, cleaved eggs and blastocyst

Why we prefer the vitrification procedure now ?

- There is no mechanical injury (extracellular crystal formation)
- Less osmotic stress for the cell
- No intracellular crystal formation
- Less labor in the laboratory daily work
- Simple protocol
- It is useful for cells like oocytes and blastocyst which have less success with slow freezing
- No need for expensive device

Is the technique of vitrification standarized to be adopted in IVF centers?

All the developmental stages are now vitrified sucessfully
These are some technical difficulties…….

a- Type and concentration of the cryoprotectant
b- Variability in the volume of the media or the carrier
c- Temperature of the solution during equilibration
d- Type of vitrification container
e- Skillness of the embryologist

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EXPERIMENTAL Study

Slow-freezing protocol

100 oocytes from 31 patients

- 66 intact oocytes
- 34 biopsied oocytes

Vitrification (Kuwayama method)

100 oocytes donated for research by 43 patients

- 67 intact oocytes
- 33 biopsied oocytes

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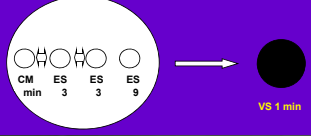
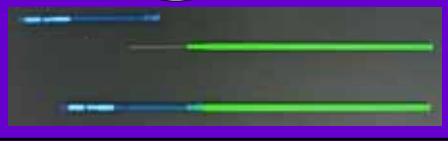
VITRIFICATION
(Kuwayama Cryotop method)

Cryoprotectants:
- 7.5% DMSO and Ethylene Glycol (EG)
- 15% DMSO and Ethylene Glycol (EG) and 0.5M sucrose.





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RE-WARMING

Solutions with different concentrations of sucrose (1M, 0.5M and 0.25M).

Thawing Solution (TS):
1 M Sucrose
10 % HSA
1 min AT 37°C

Dilution Solution 1 (DS1):
0.5 M Sucrose
10 % HSA
3 min

Dilution Solution 2 (DS2):
0.25 M Sucrose
10 % HSA
3 min

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RESULTS 1

TOTAL	PrOH Oocytes	Vitrified Oocytes
Survival	77/100 (77%)[a]	95/100 (95%)[a]

a) P=0.005

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RESULTS 2

	Intact Oocytes		Biopsied Oocytes	
	PrOH Oocytes	Vitrified Oocytes	PrOH Oocytes	Vitrified Oocytes
Survival	53/66 (80,3%)[b]	63/67 (94%)[b]	24/34 (70,6%)[c]	32/33 (96,9%)[c]

b) P=0.05

c) P=0.025

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Clinical study

Prospective randomized trial

Cycles with > 3 oocytes available for cryopreservation are randomized in

Conventional slow freezing

Vitrification

Thawing Preliminary results

	Slow freezing	Vitrification
Cycles (female age)	42 (34.5±4)	21 (36.4 ± 3.9)
Thawed eggs	181	96
Survival rate	65%	66%
Fertilization rate	82%	81%
Cleavage rate	83%	82%
Cycles transferred	35	14
Clin.pregnancies	7	6
PR/ET	20%	43%
IR	9.3	18.2
Abortions	2 (28.6%)	1 (16.7%)

- Quality is evolution
- Vitrification is a revolution

Prof. van der Elst

19.1.2007

The patient friendly approach to ART

ESHRE Campus 2009
Maribor 27-28 February

Potential "optimal" patients

- No previous ART cycles
- Normovulatory patient
- Age ≤ 40 years
- BMI < 25

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S.I.S.Me.R prospective study

Conventional approach vs IVF Lite

- conventional cycles using standard GnRH analogues protocols, conventional monitoring , cryopreservation and thawing of surplus oocytes;

- a package of three cycle with a fixed mild stimulation (clomiphene citrate 100 mg from day 3 to day 7, 150 IU of FSH on day 5,7 and 9), few monitoring from day 8 to start antagonist
- no oocyte cryopreservation.

Primary end point :
Cumulative ongoing PR over a given period of time

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Results in 12 months	Conventional	Lite
1st cycle	99	35
Fresh ongoing pregnancy	27 (27%)	10 (28%)
Thawed ongoing pregnancies	4	-
2nd or 3rd cycle	10	18
Fresh ongoing pregnancies	3 (30%)	8 (45%)
Thawed ongoing pregnancies	1	-
Cumulative OPR/ patient in one years	35% (35/99)	51% (18/35)

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