Oocyte Cryopreservation: Efficiency and Safety

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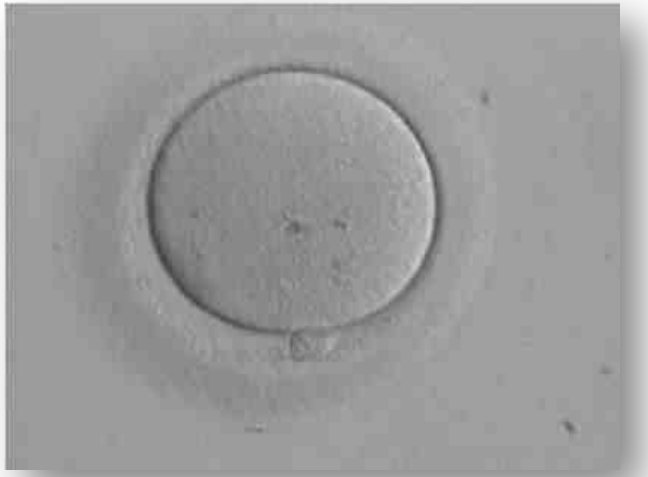
A Brief introduction

- 1978 - First IVF birth, in 1978 (UK)
- 1982 - First birth from frozen oocyte (Australia)
- Late 1980s : successful pregnancies
  by slow freeze – rapid thaw protocols
- There was a lack of progress due to
  technical concerns & low success rates



- *Due to:*
- *Extraordinary volume and poor membrane conductivity*
- *instability of microtubules & microfilaments*
- *Zona hardening*

Recent Progress

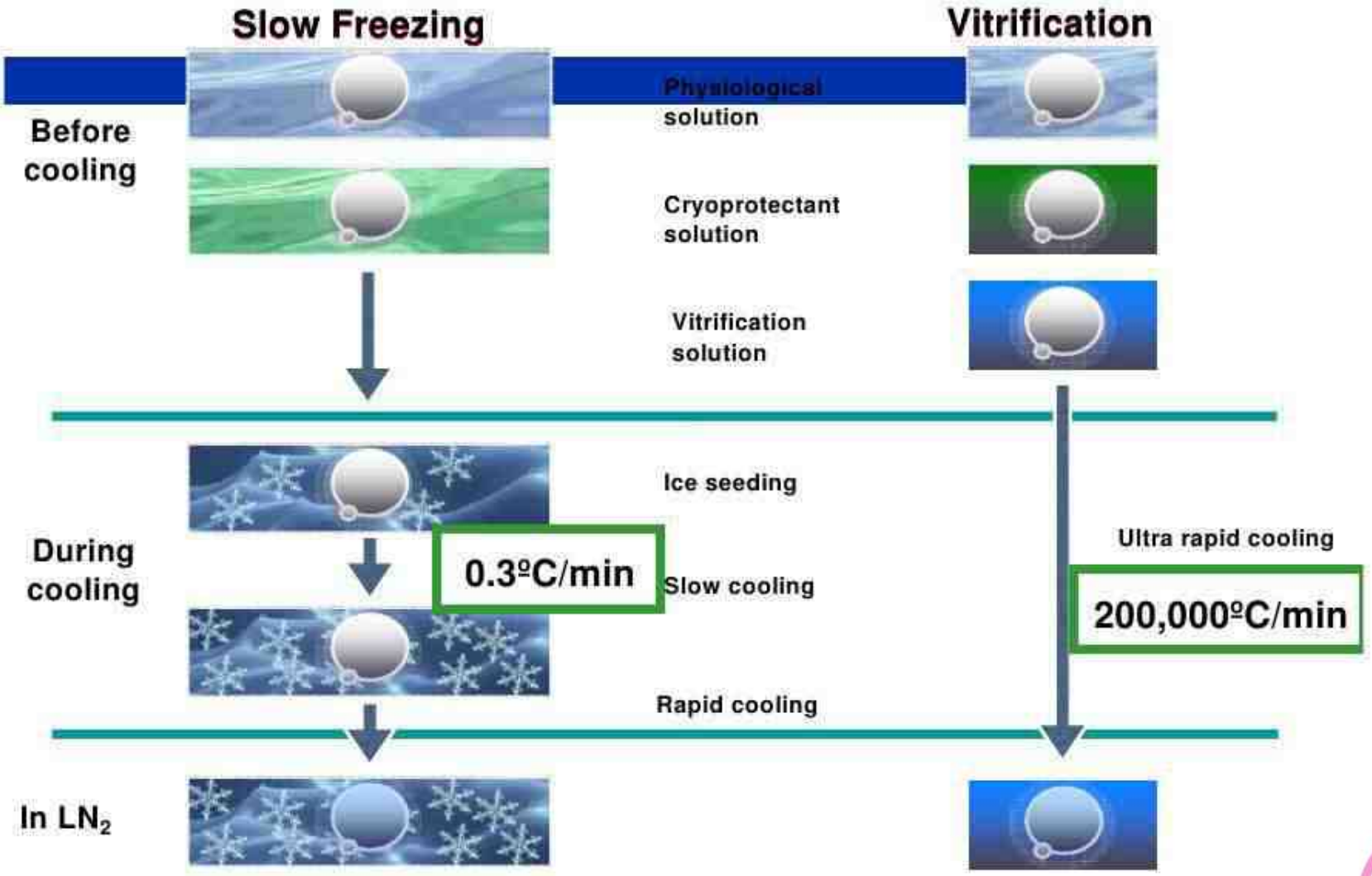
- Early 2000s:
    - Enforcing effect of legislative restrictions
    - Introduction of vitrification
    - Use of ICSI after warming of oocytes

    has improved the outcome and oocyte cryopreservation has found wider applications.

- In 2013: ASRM has lifted the experimental label on oocyte cryopreservation following four RCTs:
    - Cobo et al., 2008
    - Cobo et al., 2010
    - Rienzi et al., 2010
    - Permegiani et al., 2011

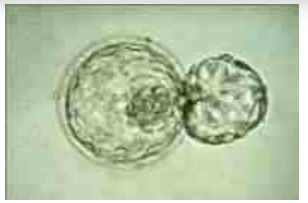
(Studies stated that vitrified/warmed oocytes can result in **similar** fertilization and pregnancy rates compared to fresh oocytes)

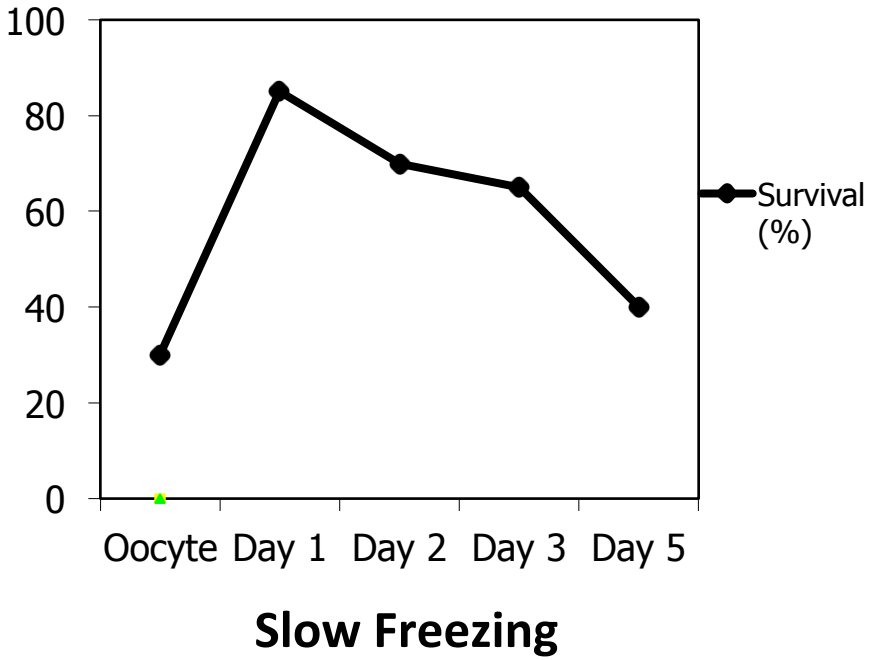
Techniques


Slow Freezing
Vitrification
Physiological solution
Before cooling
Cryoprotectant solution
Vitrification solution
Ice seeding
During cooling
0.3ºC/min
Slow cooling
Ultra rapid cooling
200,000ºC/min
Rapid cooling
In LN_2

Survival rates in the early 2000s





Slow Freezing

Oocyte cryopreservation

Vitrification

- 7.5% Ethylene Glycol
- 7.5% DMSO

Equilibriation solution(ES)

↓

15% Ethylene Glycol
15% DMSO
0.5 M Sucrose

Vitrification solution(VS)

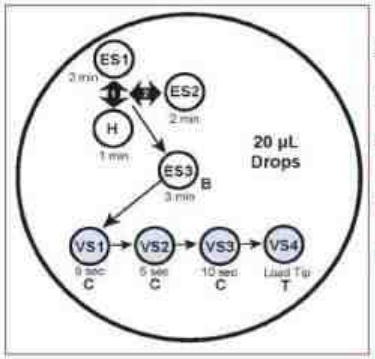
↓

(Duration of incubation in VS is critical)

Oocyte cryopreservation

Oocyte vitrification freeze protocol

Perform at Room Temperature (10 min)



1. Incubate specimen in H for 1 min
2. Merge ES1 with H for 2 min
3. Merge ES2 with H + ES1 for 2 min
4. Transfer from merged drops to BOTTOM (B) ES3 for 3 min
5. Transfer from ES3 to CENTER (C) of VS1 a proceed as shown in diagram

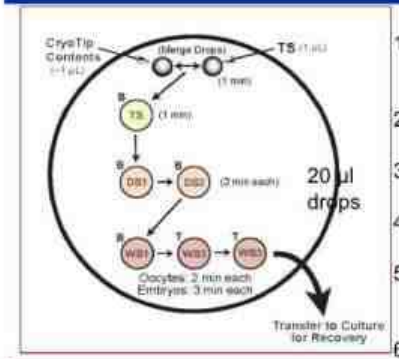
Key

H = HEPES buffered Culture Medium with protein (eg. mHTF + SSS)
ES = Equilibration Solution (3 drops)
VS = Vitrification Solution (4 drops)
B=Bottom, C=Center, T=Top

IRVINE SCIENTIFIC

Oocyte vitrification warm protocol

Perform at Room Temperature



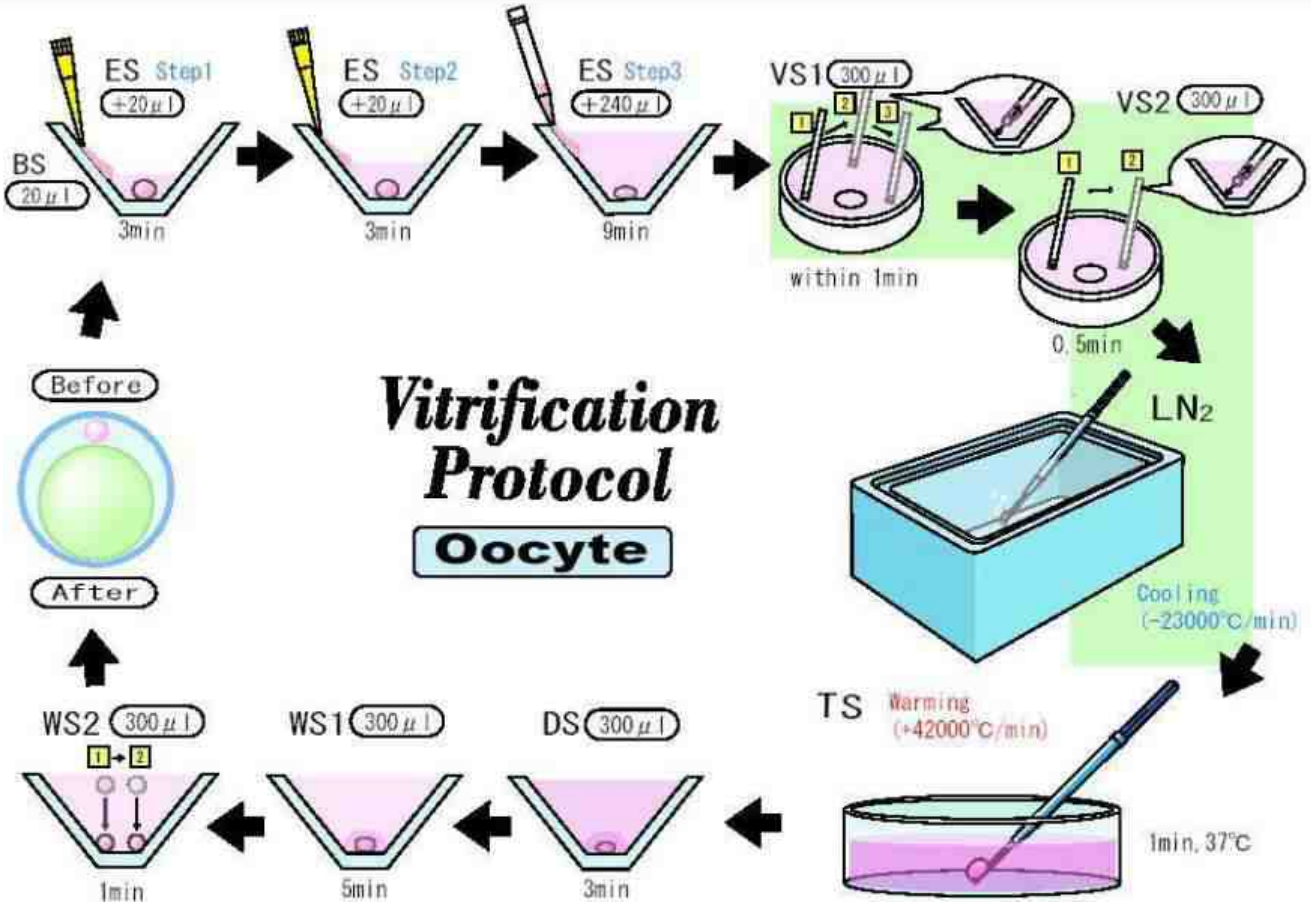
1. Rinse CryoTip™ by aspirating an equal volume (~1µl) of TS and dispensing next to CryoTip contents.
2. Merge content and rinse drop and wait 1 minute.
3. Transfer specimen(s) from merged drop to BOTTOM (B) of TS for 1 minute.
4. Transfer specimen(s) to BOTTOM of DS1 and DS2 drops for 2 minutes each.
5. Transfer Oocyte(s) (2 min) or through each WS1 (B),WS2 (T) and WS3 (T) as indicated
6. Then transfer specimen(s) to pre-equilibrated culture medium for recovery (2-3 hours) prior to subsequent manipulations.

Key

TS = Thawing Solution (1 drop)
DS = Dilution Solution (2 drops)
WS = Washing Solution (3 drops)
B=Bottom, T=Top

IRVINE SCIENTIFIC

Oocyte cryopreservation



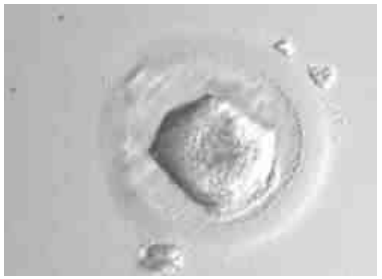
Oocyte Warming

- 1.0 M Sucrose

Thawing Solution

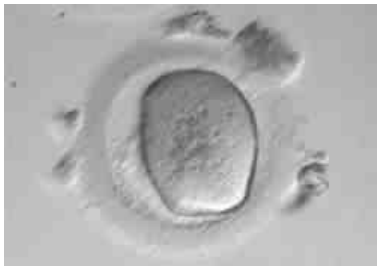
- 0.5 M Sucrose

Dilution Solution

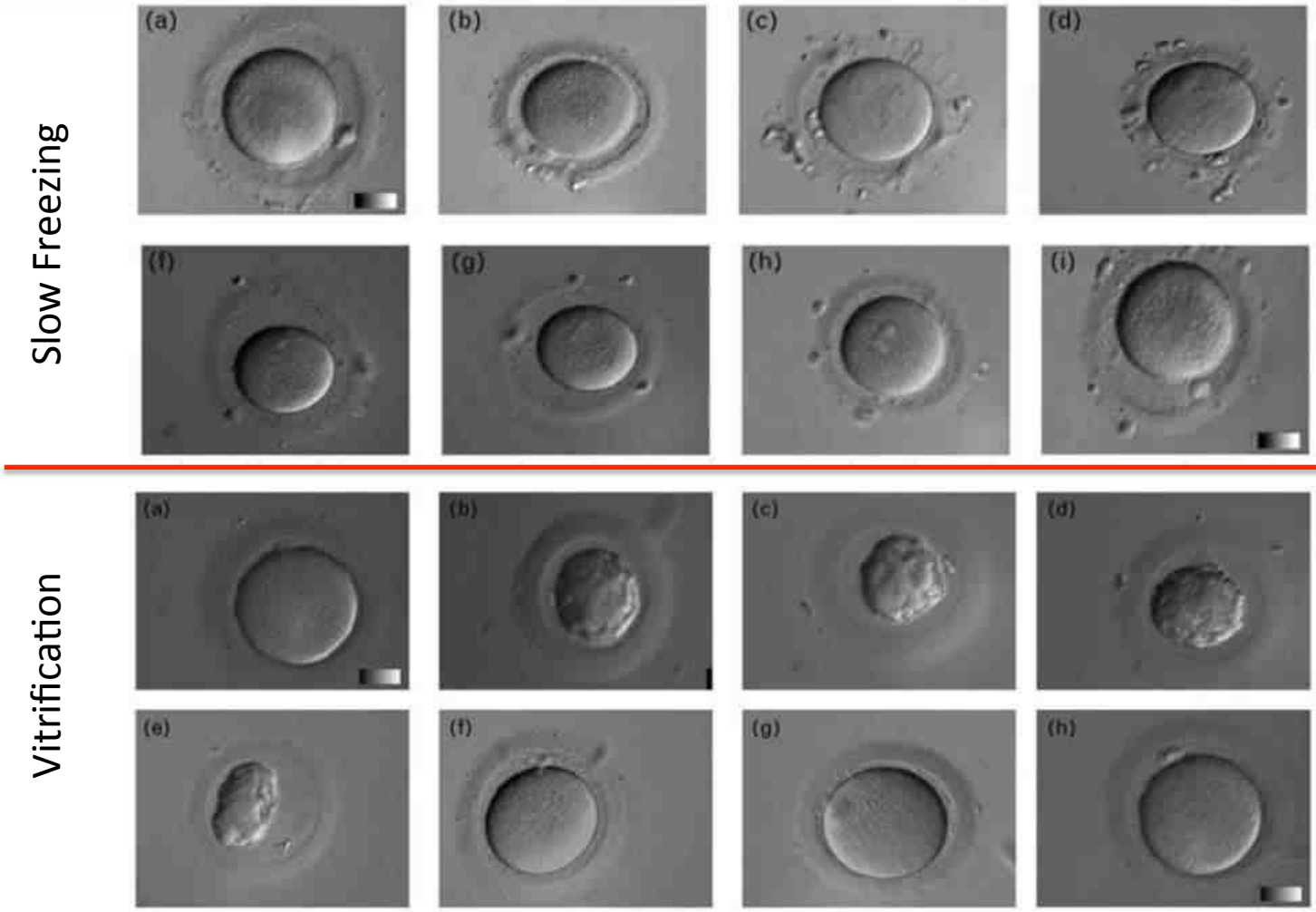


- No Sucrose

Washing Solution



Oocyte cryopreservation/warming



Cryoptotectants

Slow Freezing

1- 1.5 M

1,2 -propanediol
DMSO
Glycerol

Permeable
Low MW

0.2-1 M

Sucrose

Trehalose

Non-permeable
Low MW

High MW

Vitrification

10-20 M

Ehylene glycol
DMSO
1,2-Propanediol

} Intracellular Cryoprotectants

0.5-0.75 M

Sucrose
Trehalose

} Dehydration

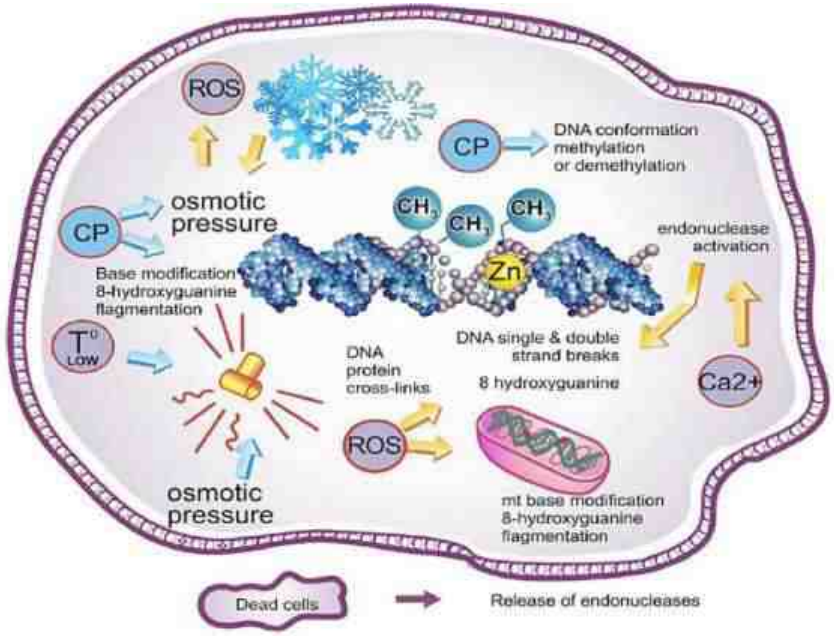
10 mg/ml

Ficoll,
PEG

} Extracellular Cryoprotectants

What is really happening during cryopreservation?

Physical factors	Fluctuation in osmotic pressure
	Mechanical forces of ice crystals
	Electrical tension between crystals
	Increased hydrostatic pressure
Chemical factors	Redistribution of the ionic components
	Changes in pH
	Phase transition in biopolymers
	Redox transformation
	Specific (toxic) and non-specific effects of cryoprotectants
	Free radicals



Kopeika et al., 2015

What is really happening during cryopreservation?

Table II Effect of different extreme factors on the DNA.

Physical or chemical factors (in non-physiological range)	Consequence for the DNA structure
Fluctuation of pH	Deamination, depurination, depyrimidation The extremes of high or low pH destabilize DNA helix and change the melting point (Williams *et al.*, 2001) Neutral or acid pH causes more chromosomal aberration during freezing–drying of mouse spermatozoa than alkaline (Kaneko *et al.*, 2003)
Temperature	Cold denaturation (Privalov, 1990; Marenduzzo *et al.*, 2001)
Reactive oxygen species	Fragmentation, base loss, single-strand breaks and double-strand breaks, production of 8-hydroxyguanine DNA–protein cross-links (Dizdaroglu and Jaruga, 2012; Jena, 2012)
Ethylene glycol	Increases hyperchromicity, ellipticity and premelt slope of chromatin. Destabilizes high melting region of polypeptide-bound DNA and the extent of higher ordered structure in model complexes and chromatin (Schwartz and Fasman, 1979). Effects melting temperature and causes the conversion from the B to the C form of chromatin (Nelson and Johnson, 1970)
Dimethyl sulphoxide	Causes DNA methylation, conformational changes in the DNA and chromatin (Nelson and Johnson, 1970) Can facilitate DNA double-break strand repair via possible conformation of heterochromatin (Kashino *et al.*, 2010)
Propylene glycol	Increases DNA methylation (Hu *et al.*, 2012)
Glycerol	Changes the conformation of DNA, has a destabilizing effect and decrease T *melting* (Nakanishi *et al.*, 1974)
Hyperosmotic stress	Results in chromosomal aberrations and DNA double-strand breaks; modulates DNA–protein binding; alters chromatin compactness (Kultz and Chakravarty, 2001); causes misfolding of proteins (Oganesyan *et al.*, 2007); induces production of reactive oxygen species (McCarthy *et al.*, 2010)

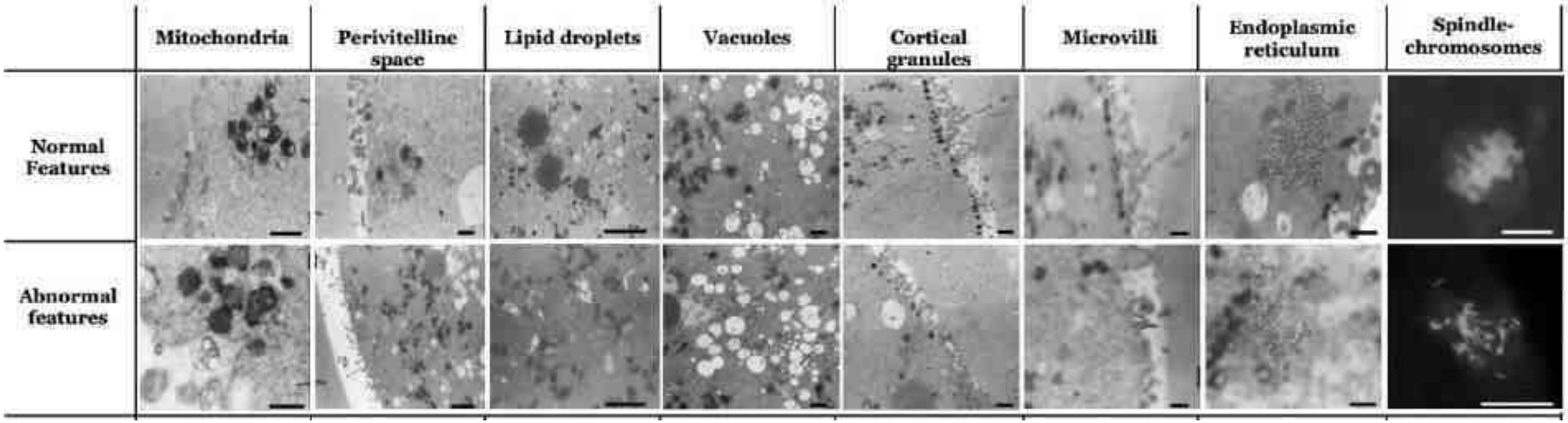
Kopeika et al., 2015

Is a frozen oocyte equal to a fresh one?

Reproductive BioMedicine Online (2016) 32, 377–387

What does the cryopreserved oocyte look like? A fresh look at the characteristic oocyte features following cryopreservation

Sayyed Morteza Hosseini *, Mohammad Hossein Nasr-Esfahani **



Is a frozen oocyte equal to a fresh one?

ARTICLE

doi:10.1038/nature11647

Towards germline gene therapy of inherited mitochondrial diseases

Masahito Tachibana[1], Paula Amato[2], Michelle Sparman[1], Joy Woodward[1], Dario Melguizo Sanchis[1], Hong Ma[1], Nuria Marti Gutierrez[1], Rebecca Tippner-Hedges[1], Eunju Kang[1], Hyo-Sang Lee[1], Cathy Ramsey[1], Keith Masterson[2], David Battaglia[2], David Lee[2], Diana Wu[2], Jeffrey Jensen[1,3], Phillip Patton[2], Sumita Gokhale[4], Richard Stouffer[1,2] & Shoukhrat Mitalipov[1,2]

Table 2 | Fertilization and embryo development of frozen rhesus oocytes

Experiment	Group	*n*	Survived after ST (%)	Survived after ICSI (%)	Fertilized (%)	Blastocysts (%)
1	Fresh oocytes	32	NA	30 (94)	29 (97)	15 (52)*
	Vitrified oocytes	26	NA	25 (96)	18 (72)	1 (6)
2	Control fresh oocytes	34	NA	33 (97)	30 (91)†	17 (57)‡
	Fresh cytoplasts Vitrified spindles	36	34 (94)	32 (94)	28 (88)†	19 (68)‡
	Vitrified cytoplasts Fresh spindles	35	35 (100)	34 (97)	17 (50)	0

* Blastocyst rate statistically different from that for vitrified oocytes ($P < 0.05$).
† Fertilized rate statistically different from that for vitrified cytoplasts with fresh spindles ($P < 0.05$).
‡ Blastocyst rate statistically different from that for vitrified cytoplasts with fresh spindles ($P < 0.05$).
Data were analysed using analysis of variance (ANOVA).
NA, not applicable.

Tachibana et al. 2013-Nature

Oocyte cryopreservation: KPIs

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

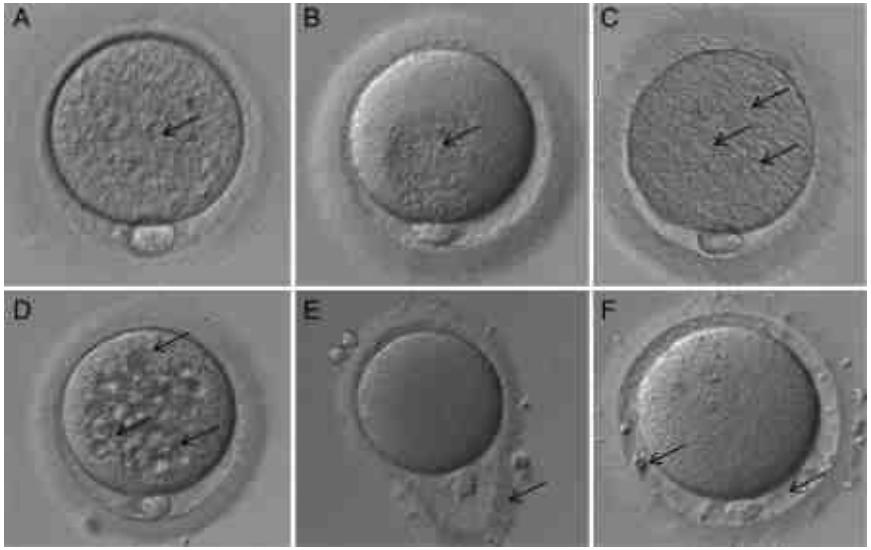
Table 1 Oocyte key performance indicator values.

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 Consensus., 2012

All oocytes are equal, but some oocytes are "more equal" than the others

"Towards a tailor-made oocyte cryopreservation"



- Modifications in protocols and handling should be needed based on the "nature" of the oocytes (high or poor quality)
- Cryopreservation media should be improved in order to minimize the stress (antioxidants, free radical chelators)

Oocyte cryopreservation: Indications

Oocyte cryopreservation: where are we now?

Catrin E. Argyle[1], Joyce C. Harper[2,*], and Melanie C. Davies[3]

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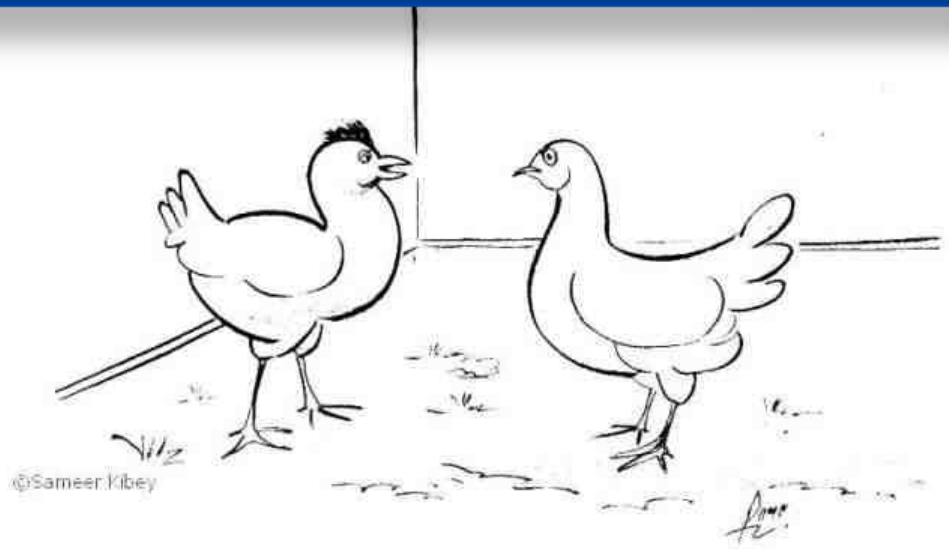
- Fertility preservation in cancer patients
- Oocyte cryopreservation for medical reasons
    - *Endometriosis, autoimmune diseases etc.*
- Oocyte Donation
- Elective oocyte cryopreservation (social freezing)
    - *Age-related fertility decline*
- "Emergency" oocyte cryopreservation

Argyle et al., 2016

Social egg freezing



I am really happy at this poultry. I heard the employer freezes our eggs too!

Concerns

- Procedure-associated risks?
  - Proper consenting?

- 1468 women undergoing elective oocyte cryopreservation for non-oncologic reason
- 137 returned to use them – pregnancy rates were found to be age-dependent
- Optimal number of stored MII oocytes should be at least 8 – 10.

Cobo et al., 2015

Oocyte donation from vitrified oocytes

Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate

Ana Cobo, Ph.D., Nicolás Garrido, Ph.D., M.Sc., Antonio Pellicer, M.D., and José Remohí, M.D.

IVI-Valencia, Institut Universitari IVI, Valencia, Spain

Patient(s): Recipients of vitrified oocytes (January 2007–March 2013), including all the warming procedures (n = 3,610) and all the donations made during the same period (n = 3,467).

Intervention(s): None.

Main Outcome Measure(s): Survival rate per warming procedure, cumulative delivery rates (CDR) per single donation cycle, oocyte-to-baby rate, and cumulative live birth rate (CLBR) per oocyte consumed.

Result(s): Oocyte survival rate was 90.4%. It was not possible to develop a predictive model for survival owing to the lack of prognostic value of the studied variables. Implantation, clinical, and ongoing pregnancy rates per donation cycle were 39.0% (95% confidence interval [CI], 37.8–40.5), 48.4% (95% CI, 46.7–50.1), and 39.9% (95% CI, 38.3–41.5), respectively. Statistical differences were found when comparing blastocysts versus day 3 ETs (42.5%; 95% CI, 40.4–45.2 vs. 37.5%; 95% CI, 35.3–39.7 ongoing pregnancy rate). The CDR/donation cycle, including cryotransfers, was 78.8% (95% CI, 73.5–84.1). The oocyte-to-baby rate was 6.5%. CLBR increased progressively according to the number of oocytes consumed.

TABLE 4

Cumulative live birth according to the number of oocytes consumed.

No. of oocytes consumed	CLBR (%)	95% CI
5	6.1	5.3–7.0
10	39.4	37.5–41.3
12	53.5	51.4–55.5
15	67.5	65.4–69.8
20	80.5	78.3–82.7
25	85.4	83.2–87.6
30	89.9	87.6–92.1
35	94.8	92.3–97.2
40	95.5	93.0–97.9
43	97.3	94.9–99.7

Note: The increase in CLBR was of approximately 3.9% per additional oocyte from 5–10 oocytes; 5.6% from 10–15 oocytes; 2.6% from 15–20 oocytes; 1.0% from 20–25 oocytes; 0.9% from 25–35 oocytes; 0.2% from 35–40 oocytes, and 0.1% when >40 oocytes were consumed.

Cobo. Six years of egg banking for ovum donations. Fertil Steril 2015.

Cobo et al., 2015

Follow up on children born after cryopreservation of oocytes

- To date, there are no long-term follow up studies for children from cryopreserved oocytes and most data are from case reports and retrospective studies.

- Mean birthweight and incidence of congenital abnormalities were similar in infants born through regular IVF (n=200 infants)

  (Chian et al. 2008)

- Incidence of congenital abnormalities were similar after slow freezing or vitrification (n=936 infants)

  (Noyes et al. 2009)

Noyes, et al. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. RBM Online 18:769-776, 2009.

Parameter	Slow-Freeze	Vitrification	Both	Total
# Embryo transfers	2003	844	19	2866
# live born babies	532	392	12	936
Birth Defects (Incidence)	6 (one in 89)	6 (one in 65)	0	12 (one in 78)

All birth anomalies*	Approximate incidence in natural conception births	Incidence in total of 936 oocyte cryopreservation births (n)
	One in 33	One in 78 (12)

* Skin haemangioma, cardiac defects, neural tube defects, cleft lip/palate, clubfoot, Arnold-Chiari syndrome, chroanal atresia, biliary atresia, Rubinstein-Taybi syndrome

Conclusions: Where are we now?

- Oocyte cryopreservation has now been an established technology with a wide range of indications.
- Vitrification has now been the method of choice.
- However, there is an urgent need to monitor the medical indications and technical approaches on clinical outcome and long term follow up of children after oocyte cryopreservation/ warming.
- More and extensive research is needed to monitor the possible effects of cryopreservation on DNA (genetic & epigenetic changes)