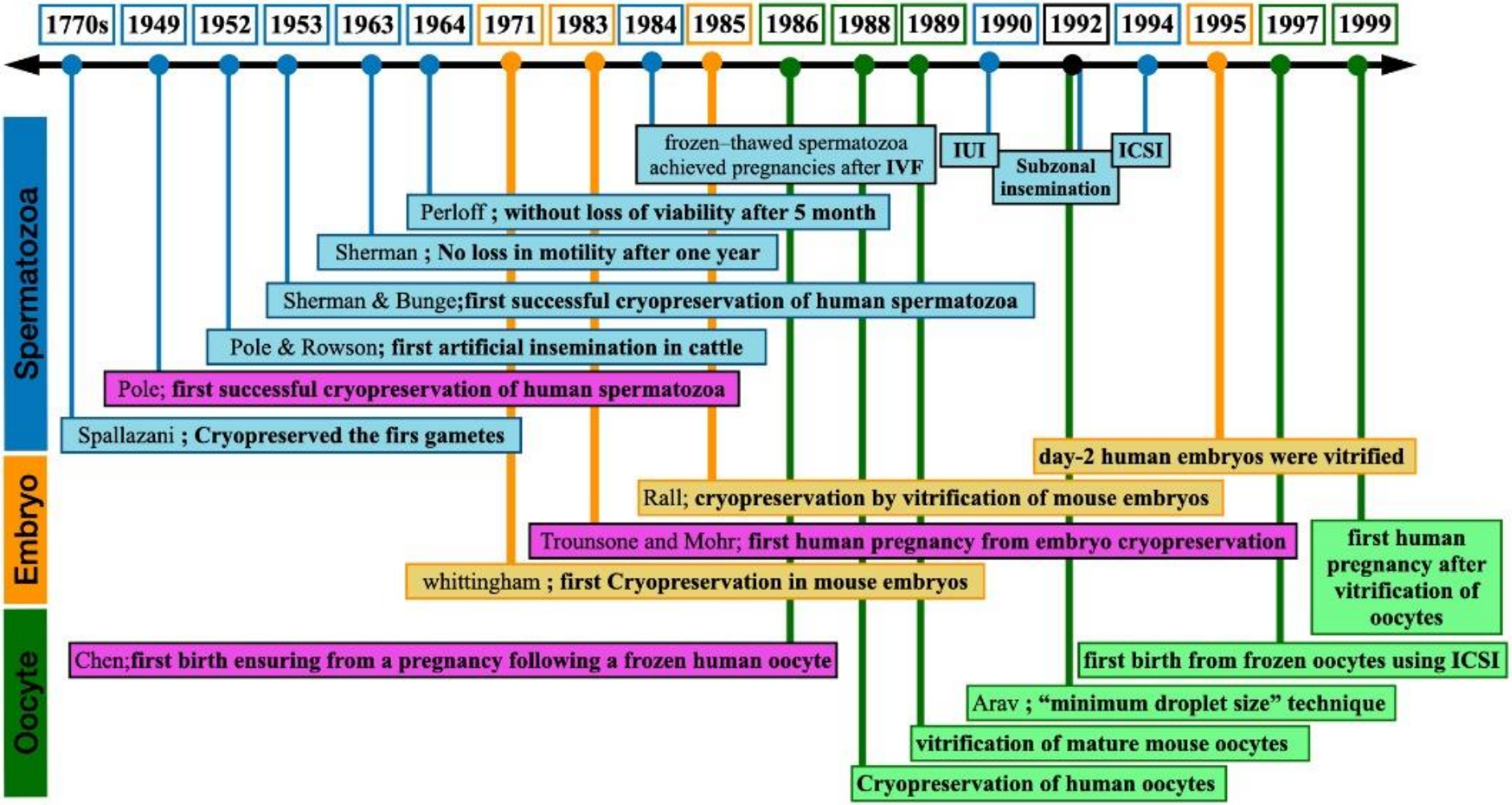

بسم الله الرحمن الرحيم

Royan Institute

Cryopreservation in ART

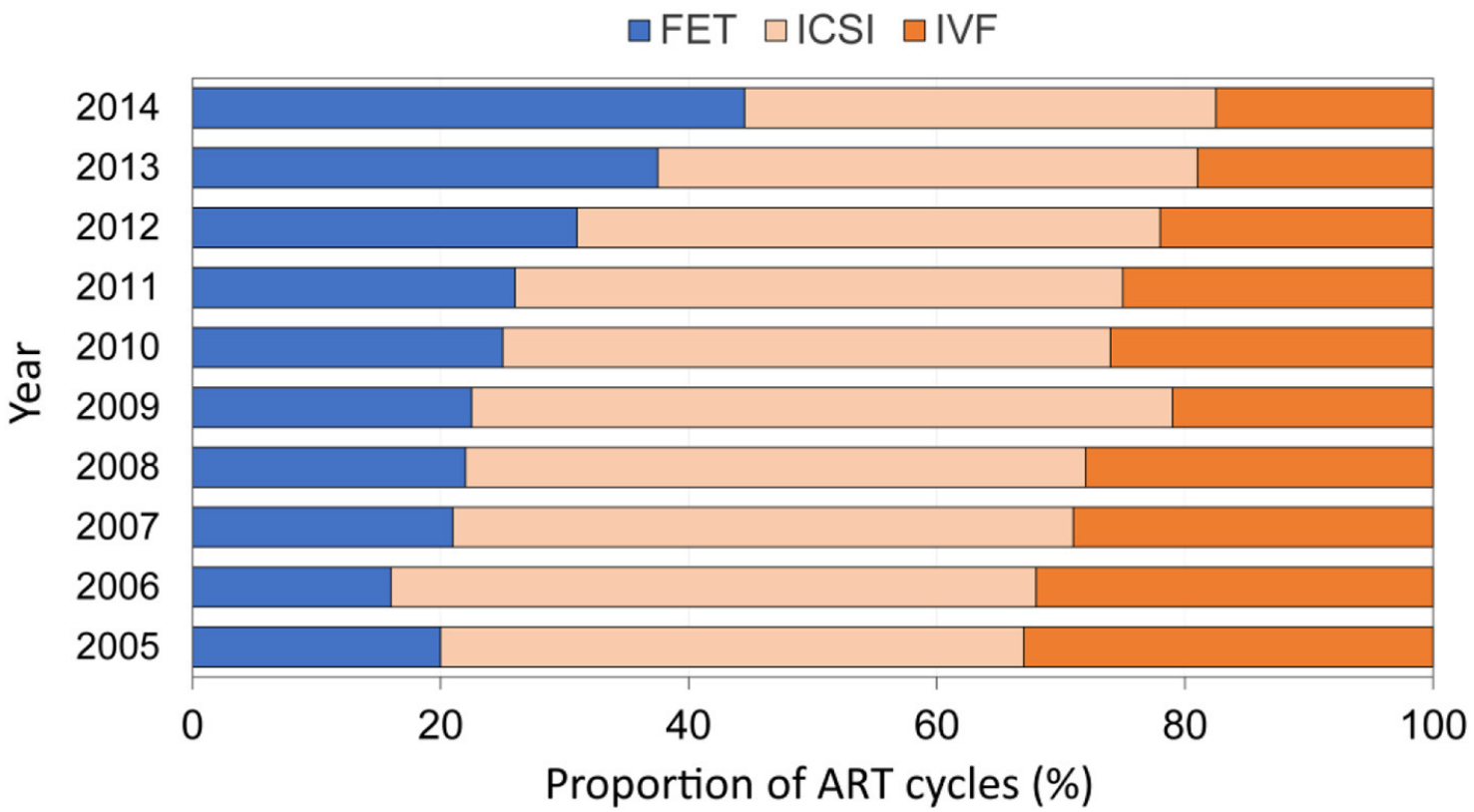
History


1770s
1949
1952
1953
1963
1964
1971
1983
1984
1985
1986
1988
1989
1990
1992
1994
1995
1997
1999
Spermatozoa
Embryo
Oocyte
frozen–thawed spermatozoa achieved pregnancies after IVF
IUI
Subzonal insemination
ICSI
Perloff ; without loss of viability after 5 month
Sherman ; No loss in motility after one year
Sherman & Bunge;first successful cryopreservation of human spermatozoa
Pole & Rowson; first artificial insemination in cattle
Pole; first successful cryopreservation of human spermatozoa
Spallazani ; Cryopreserved the firs gametes
day-2 human embryos were vitrified
Rall; cryopreservation by vitrification of mouse embryos
Trounsone and Mohr; first human pregnancy from embryo cryopreservation
first human pregnancy after vitrification of oocytes
whittingham ; first Cryopreservation in mouse embryos
Chen;first birth ensuring from a pregnancy following a frozen human oocyte
first birth from frozen oocytes using ICSI
Arav ; "minimum droplet size" technique
vitrification of mature mouse oocytes
Cryopreservation of human oocytes

Cryopreservation in ART

- Societal changes and the increasing desire and opportunity to preserve fertility have increased the demand for effective assisted reproductive technologies (ART) and have increased the range of scenarios in which ART is now used.

Cryopreservation in ART



The data from the US for CDC and Prevention show that the proportion of embryo transfers derived from freeze-all cycles is increasing—from around 20% in 2005 to almost 50%/ in 2015.

Indications for Cryopreservation in ART Practice

Elective

- Oocyte donation,oocyte banking
- Social freezing
- Clinical oocyte freezing
- Transgender

Non elective

- Medical oocyte freezing
- Incidental oocyte freezing

Indications for Cryopreservation in ART Practice

Elective

- Preimplantation genetic testing (PGT)
- Patient's or physician's preference

Non elective

- Elevated progesterone
- Avoidance of OHSS

Social egg freezing

Social egg freezing

- In 1990 the average age of women at their first marriage was 22 years and that of men was 24.7 years, in 2010 women got married at the age of 28.7 years and men at the age of 31.4 years.”

Social egg freezing

For many women today, it is not cancer but the simple passage of time that robs them of their chance of motherhood.

often lead them to delay trying to start a family until their late thirties, by which time the chance of success is very low.

Social egg freezing

- Use of frozen thawed sperm has been in clinical use for over 50 years, and banking sperm has been routinely offered to men, usually before gonadotoxic treatments, but also in many cases, practiced as a ‘‘safety policy’’ before a vasectomy.

Social egg freezing

- Freezing methods for women's egg have required a much longer time to achieve a comparable effective clinical standard. Only recently, with the development of vitrification of oocytes, the clinical standard was recognized, and since 2013 when the label **"experimental"** was removed, the freezing of oocytes could be regarded as an established method, and its use extended into clinical practice for fertility preservation and **"social freezing"**

Non-medical egg freezing has only been available for about the last 7 years.

Social egg freezing

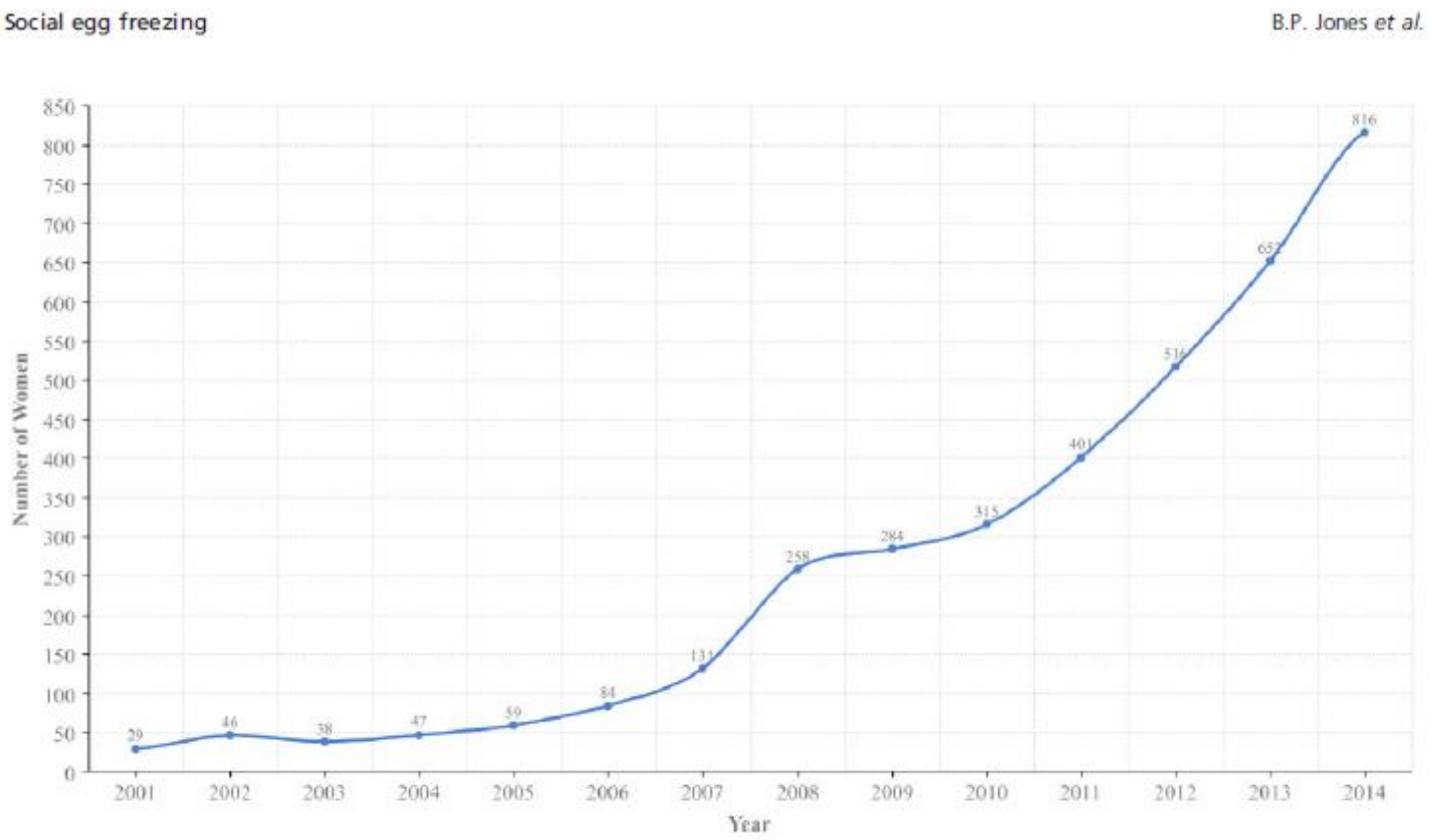


Figure 3. Number of women undergoing elective oocyte cryopreservation in the UK in 2014. Based on data reported in the HFEA trends and figures 2014 data sheet on egg freezing (7). [Color figure can be viewed at wileyonlinelibrary.com].

Social egg freezing

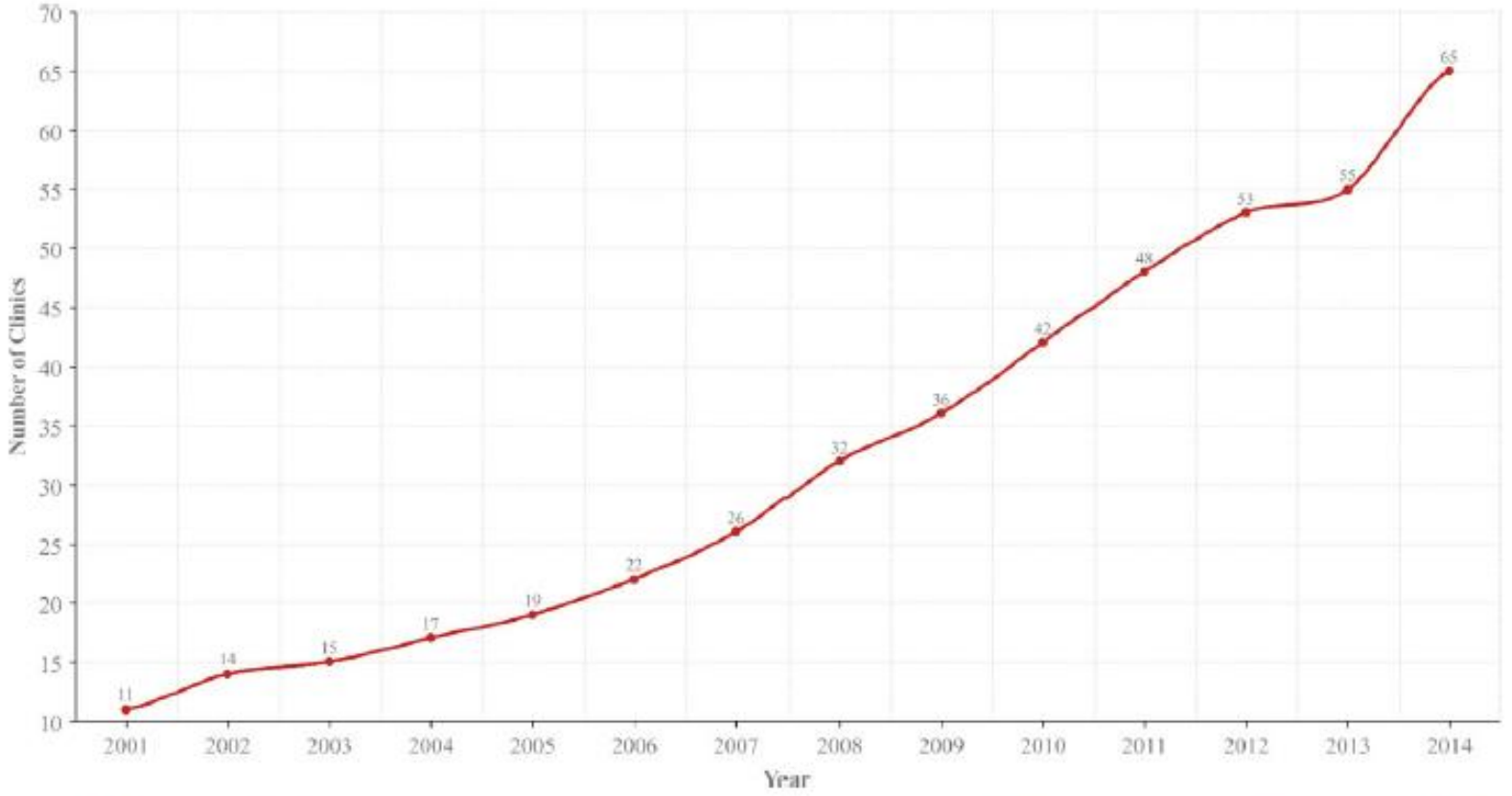


Figure 4. Number of fertility clinics offering elective oocyte cryopreservation in the UK in 2014. Based on data reported in the HFEA trends and figures 2014 data sheet on egg freezing (7). [Color figure can be viewed at wileyonlinelibrary.com].

Social egg freezing

- Women at age 40 face a 40 % chance of miscarriage if they can get pregnant at all, and by the age of 45, the risk of miscarriage is 75 %.
- Donor eggs are not an option for many because of supply constraints and ethical and cultural concerns.

Social egg freezing

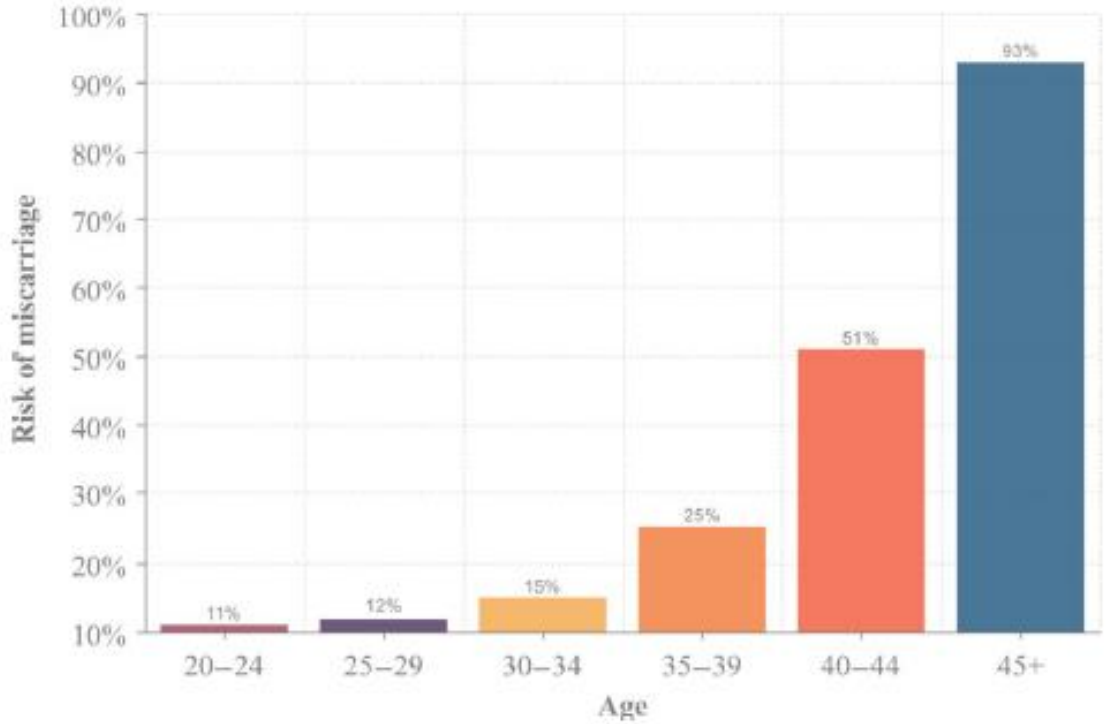


Figure 1. Relation between miscarriage and advancing age. Graph based on the data from Nybo Anderson et al. (4) (page 1710, paragraph 4, lines 4–6). [Color figure can be viewed at wileyonlinelibrary.com].

It seems that between 30 and 35 would be the ideal time: not too soon, but not too late because the quality decreases very rapidly each year after 35 (Mesen 2015).

In a more general sense, oocytes cryopreservation may be regarded as a step towards decreasing differences between genders regarding choice in reproduction

can embryos be frozen twice?

607 RBMO VOLUME 43 ISSUE 4 2021

RBMO

ARTICLE

Pregnancy potential and perinatal outcomes of embryos cryopreserved twice: a case–control study

Outcome	Case (*n* = 89)	Control (*n* = 304)	*P*-value
Pregnancy (%)	33 (37.1)	131 (43.1)	0.34
Clinical pregnancy (%)	28 (31.5)	112 (36.8)	0.35
Miscarriage[b] (%)	4 (4.5)	12 (3.9)	0.77
Extrauterine pregnancy (%)		2 (0.7)	
Termination of pregnancy[a] (%)		1[d] (0.3)	
Biochemical pregnancy[a] (%)	5 (5.6)	12 (3.9)	0.81
Live birth (%)	24 (27.0)	97 (31.9)	0.35
Singleton (*n*)	23	94	
Twin (*n*)	1	3	
Newborns (*n*)	25	100	
Gestational age[c] (weeks)	40 (39, 41)	39 (38, 40)	0.065
Preterm deliveries[b] (<37 gestational weeks, %)	1 (4.2)	10 (10.3)	0.69

Weight[c] (g, singleton pregnancies)	3730 (3500, 4050)	3490 (3150, 3900)	0.064
Weight group[a]			
AGA (%)	25 (100.0)	90 (90.0)	
LGA (%)	0	8 (8.0)	
SGA (%)	0	2 (2.0)	
Sex, boys[a] (%)	12 (48.0)	45 (45.0)	0.77

AGA = appropriate for gestational age; LGA = large for gestational age; SGA = small for gestational age.

[a] Analysed with chi-squared test.

[b] Analysed with Fisher's exact test.

[c] Analysed with Wilcoxon rank-sum test; results presented as median (quartiles, Q1, Q3).

[d] Pregnancy terminated due to aneuploidy.

Conclusions: Uncompromised live birth rates and neonatal outcomes may be expected after the transfer of twice-cryopreserved embryos. To avoid embryo wastage and transfer of multiple embryos, good quality surplus embryos from FET cycles may be cryopreserved again by vitrification.

ELSEVIER

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Outcomes of blastocysts biopsied and vitrified once versus those cryopreserved twice for euploid blastocyst transfer

Tyl H Taylor [a,b], Jennifer L Patrick [a], Susan A Gitlin [c], J Michael Wilson [a], Jack L Crain [a], Darren K Griffin [b,*]

Characteristic Intervention	Group 1	Group 2			
	Biopsy/vit/warm (n = 85)	Vit/warm/biopsy/revit/rewarm (n = 9)	Slow/thaw/biopsy/vit/warm (n = 6)	Biopsy/vit/warm/rebiopsy/revit/rewarm (n = 2)	Total (n = 17)
Patient age at retrieval (years)	35.6 ± 3.9	35.3 ± 4.8	34.1 ± 5.5	39.0 ± 2.8	35.3 ± 4.9
Cryopreserved blastocysts thawed/warmed	116	12	9	3	24
Blastocysts survived	114 (98.3)[a,b]	12 (100.0)	7 (77.8)[b]	2 (66.7)	21 (87.5)[a]
Transfers					
Total transfers	85	9	5	2	16
Total embryos transferred	113	12	7	2	21
Embryos per transfer	1.3 ± 0.6	1.3 ± 0.5	1.2 ± 0.8	1.0 ± 0.0	1.3 ± 0.5
Biochemical pregnancies/transfer	58 (68.2)	6 (66.7)	4 (80.0)	0 (0)	10 (62.5)
Clinical pregnancies/transfer	52 (61.2)	5 (55.6)	4 (80.0)	0 (0)	9 (56.3)
Fetal cardiac activity/transfer	49 (57.6)	5 (55.6)	3 (60.0)	0 (0)	8 (50.0)
Implantation/per transfer	66 (58.4)	6 (50.0)	5 (71.4)	0 (0)	11 (52.4)
Live birth or ongoing pregnancy/transfer	61 (54.0)	6 (50.0)	4 (57.1)	0 (0)	10 (47.6)

Values are mean ± SD, *n*, or *n* (%).
Slow = slow freezing; vit = vitrification.
[a] $P = 0.0354$.
[b] $P = 0.0258$.

Cryopreservation in ART

- **Improvements in cryopreservation techniques (vitrification)**
- **Efficient ovarian stimulation protocols**

Elective frozen embryo transfer (eFET)

Practical tips for vitrification

- ✓ Pre-equilibrate all media to the correct temperature prior to dispensing, and invert the vials immediately before use to make sure that the solutions are fully mixed.

- ✓ Label each vitrification device fully prior to use. **Remember that timing is crucial to effective vitrification and warming,** and make sure that everything is “ready to go” prior to starting the procedure.

Practical tips for vitrification

- ✓ Prepare a LN2 bath and place it next to the flowhood, ready for plunging the loaded vitrification devices.
- ✓ The vessel should be a properly functioning container dedicated for the purpose of holding LN2.
- ✓ Portable insulated containers used for media transport/deliveries are not designed for use as LN2 baths, and using them for this purpose is highly inadvisable.
- ✓ Any leak of LN2 could have disastrous consequences.
- ✓ Place the LN2 bath on a flat secure surface; do not use a chair

Practical tips for vitrification

- Carry out all vitrification/warming procedures in an area that will **remain free of distraction throughout**.
- Adhere to the correct timings strictly, using a timer. Using two separate timers can prevent the loss of vital seconds in re-setting a single timer. It is also helpful to have a second embryologist to assist with the timings, etc.
- If a straw sealer is used, make sure that it is switched on and ready to use before starting the vitrification process.

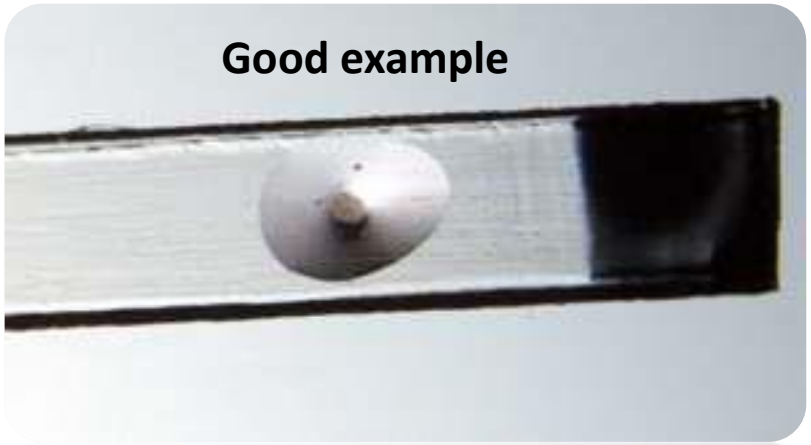
Practical tips for vitrification

- Some media companies recommend using **1–2 ml solution** volumes for each vitrification/warming event, but **100–200 µl** droplets are equally effective, provided that the oocyte/embryo is full equilibrated in each solution.
- Larger volumes for warming (**800–1000 µl**) have the advantage that the vitrification device can be easily submerged into a large volume, making removal of the oocyte/embryo easier.

Practical tips for vitrification

Reducing the volume of the sample to a minimum when placing embryos onto the vitrification device is thought to reduce potential CPA toxicity and osmotic damage;

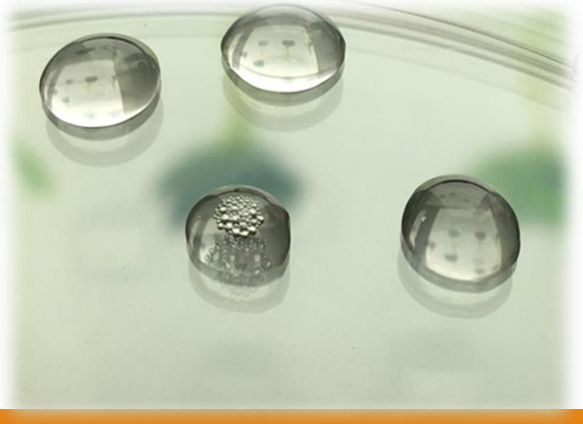
- Volume of 1–2 µl per sample, The volume can be decreased even further to leave only a "film" around the embryo..
- Removal of almost all of the fluid prior to plunging into LN2 can result in poorer survival rates in some circumstances, and leaving at least 1 µl around the embryos may be preferable



Practical tips for vitrification

- Ensure that there are no air bubbles on the surface of the initial warming solutions, as embryos tend to adhere to bubbles; this will hinder full submersion into the solution, and also affect the precise timing of the warming process.

- The speed of warming (around 20000 °C/min) is considered to be more important in avoiding lysis than is the cooling speed. If warming is too slow, the intracellular CPA concentration is too low to prevent ice from re-crystallizing, and the supercooled liquid forms lethal ice crystals.



Blastocoele collapse (Assisted Shrinkage)

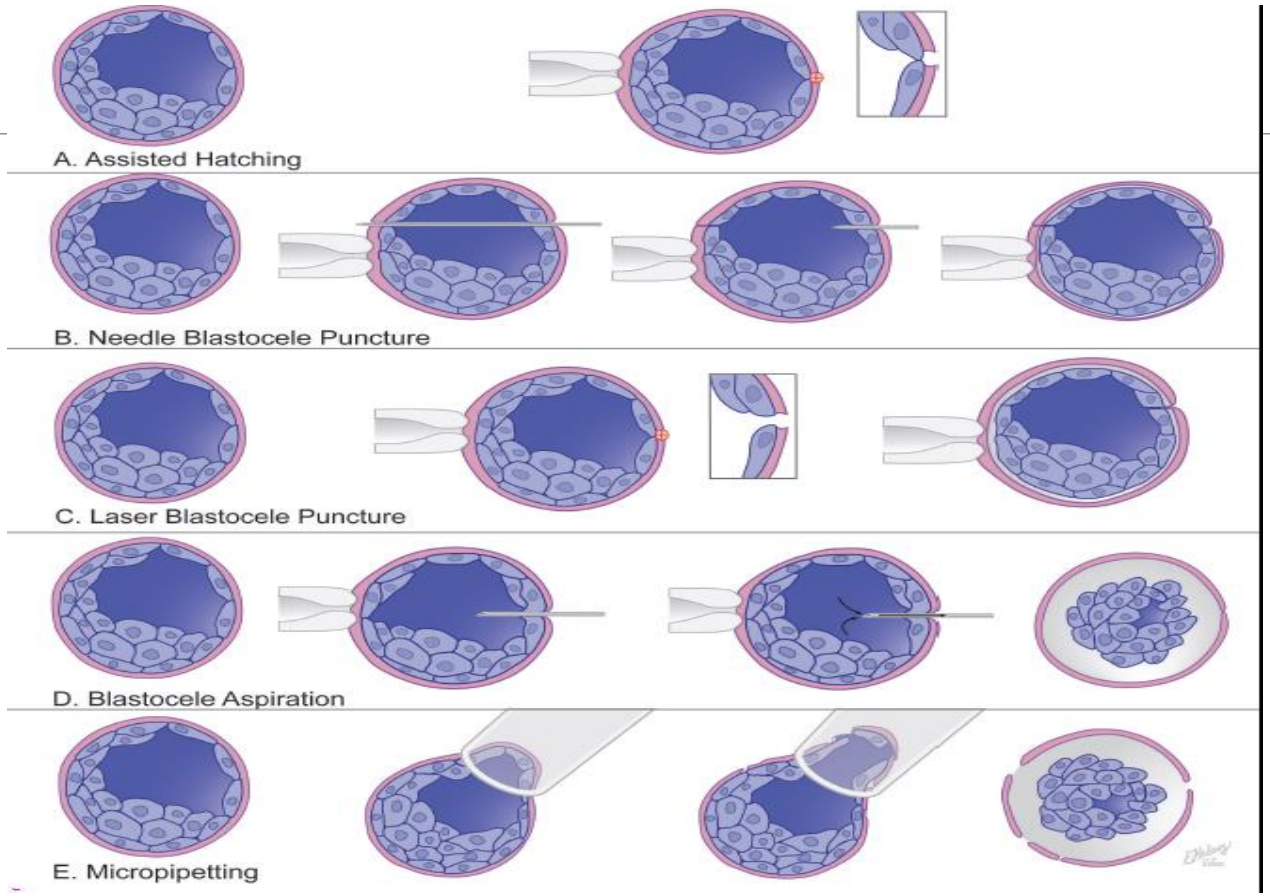
Much attention has been paid to ***the volume of the blastocoele*** prior to vitrification and its effect on the overall success of vitrification.

A ***negative correlation*** between blastocelic volume and outcome measures has been attributed to an increased likelihood of intracellular ice formation in an inadequately dehydrated blastocoele .

Consequently, a process called ***assisted shrinkage*** was developed to reduce blastocelic volume prior to vitrification.

.

Blastocoele collapse (Assisted Shrinkage)



There were no statistical differences in survival, implantation and clinical pregnancy rates **between blastocysts that had undergone laser pulse opening or micro-needle** puncture .

Practical tips for vitrification

Embryo survival may not be immediately obvious after warming, and survival/ morphology is routinely assessed after a minimum period of 2 hours in culture.

This is particularly important in the case of blastocysts, which may appear collapsed immediately after warming but will re-expand within 2 hours when cultured under optimal conditions.

Blastocyst freezing

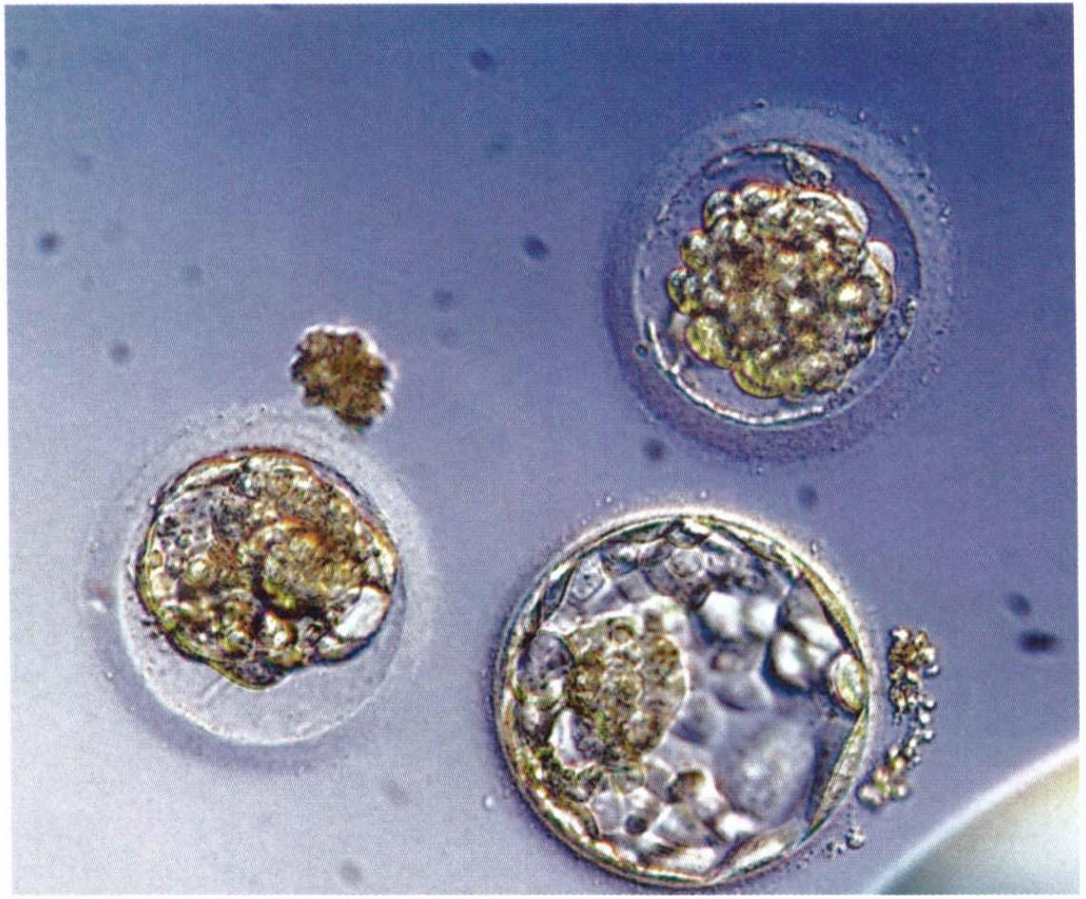


FIGURE 9-1. Three thawed blastocysts with one collapsed (*top*), one partially reexpanded (*left*), and a third embryo (*bottom right*) fully reexpanded. Note the differences in zona thicknesses.

Practical tips for vitrification

- ✓ Theoretically, several oocytes or embryos can be cryopreserved in a single straw or on a single vitrification carrier.
- ✓ Guidelines for good practice suggested by the Association of Clinical Embryologists in the UK recommend that no more than two embryos should be loaded per device.
- ✓ Loading only one embryo per device presents less potential risk to the embryo(s).
- ✓ Two embryos from a single patient cryopreserved on two devices also allows them to be stored in separate dewars, reducing the risk in case of a dewar failure.

Practical tips for vitrification

- ✓ Participation in workshops provided by companies that supply vitrification media can be helpful, where experts can demonstrate optimal use of specific devices and media.
- ✓ Visiting a lab that has a good vitrification program can also be helpful.
- ✓ The vitrification technique can be practiced using unfertilized oocytes or abnormally fertilized embryos (both with patient consent) before it is applied in treatment cycles.

Cryostore Management

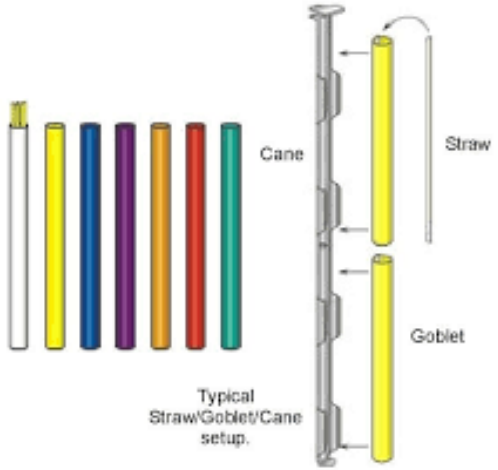
- ✓ The capacity of a cryostore depends on the freezing strategy of the clinic and its activity, i.e. the number of cycles with potential freezing performed per year.
- ✓ The number of dewars/ vapor stores can then be predicted, always allowing for a spare dewar for emergency use.


CE
0086

Cryostore Management

- Colored goblets help in keeping track of samples
- They can be arranged in alphabetical order, as shown here: aqua, black, blue, brown, green, gray, orange, pink, purple, tangerine, yellow, or in the order of the rainbow/white light spectrum.
- A canister that has just been removed from liquid nitrogen,

containing straws that are distributed between different colored goblets.



Cryostore Management

All dewars must be inspected regularly for damage, making sure that the vacuum is functional. Visual inspection of the outside can sometimes reveal “cold spots,” which may indicate a fault with the vacuum.

Cryostore Management

- ✓ Failure to check dewars can have disastrous consequences for cryopreserved material.
- ✓ Dewars should be equipped with an alarm that is triggered by low LN2 levels, either via a platform base that registers weight change, or with a system that monitors temperature inside the dewar

An Ohio fertility clinic said that the remote alarm system on its storage tank was turned off, so it didn't know that the temperature had fluctuated, and that the consequences were worse than it initially thought — all 4,000 eggs and embryos in the cryofreezer are likely nonviable.

Cryostore audit

- ✓ It is important to maintain contact with patients who have their gametes/embryos in storage, and confirm that storage is in accordance with their wishes. The regulatory bodies of many countries produce their own consent forms to cover a defined period of storage.
- ✓ In-house consent forms can act as legal contracts between the patients and the clinic, clearly defining the action to be taken if the patients fail to maintain contact with the clinic or cannot be contacted.

HFEA (00)6 FORM FOR CONSENT TO STORAGE AND USE OF SPERM AND EMBRYOS

N.B. Do not sign this form unless you have received information about these matters and have been offered counselling. You may vary the terms of this consent or withdraw this consent at any time except in relation to sperm or embryos which have already been used. Please insert numbers or tick boxes as appropriate.

Full name (block capitals):

Any other name by which you have been known:

I. USE

a. I hereby consent to the use of my sperm for the following purposes:

i. in treating a named partner YES☐ NO☐
Full name of partner:

ii. in treating others YES☐ NO☐

iii. in any project of research YES☐ NO☐

Please state any particular conditions as to use:

b. I hereby consent to the use of my sperm to fertilise egg(s) in vitro and to the use of embryo(s) developed from these egg(s) for the following purposes:

i. in the treatment of myself together with a named partner YES☐ NO☐

Full name of partner:

ii. in treating others YES☐ NO☐

iii. in any project of research YES☐ NO☐

Please state any other conditions as to use (eg on the use of particular embryos):

Signature: _____ Date: DAY ☐☐ MONTH ☐☐ YEAR ☐☐

II. STORAGE

a. I hereby consent to the storage of my sperm:

Storage period in years Maximum (10 years)* YES☐ NO☐ If less please state YEARS☐

*Centres are allowed to store sperm for longer periods for limited uses only.

b. I hereby consent to the storage of embryo(s) developed in vitro from egg(s) fertilised with my sperm:

Storage period in years:

Five years YES☐ NO☐ Ten years YES☐ NO☐ More than ten years YES☐ NO☐

If less than five years or some other period please state the number of years: YEARS ☐

I understand that consent to storage of more than five years must be accompanied by a completed HFEA(96)8 form which has been signed by a registered medical practitioner.**

**This does not apply to donors.

c. If I die or become mentally incapacitated my sperm or the embryo(s) developed in vitro from egg(s) fertilised with my sperm should:

	SPERM		EMBRYOS	
i. be allowed to perish	YES☐	NO☐	YES☐	NO☐
ii. continue in storage for the purpose given in 1a. (for sperm) and 1b. (for embryos) above	YES☐	NO☐	YES☐	NO☐
iii. continue in storage for other purposes (please specify below)	YES☐	NO☐	YES☐	NO☐

d. Any other conditions of storage (eg for particular embryos).
Please state:

e. I understand that unless they are used beforehand embryo(s) developed in vitro from egg(s) fertilised with my sperm will have to be allowed to perish at the end of the storage period specified at IIb.

Signature: _____ Date: DAY ☐☐ MONTH ☐☐ YEAR ☐☐

HFEA (00)6 FORM FOR CONSENT TO STORAGE AND USE OF SPERM AND EMBRYOS

N.B. Do not sign this form unless you have received information about these matters and have been offered counselling. You may vary the terms of this consent or withdraw this consent at any time except in relation to sperm or embryos which have already been used. Please insert numbers or tick boxes as appropriate.

Full name (block capitals):

Any other name by which you have been known:

I. USE

a. I hereby consent to the use of my sperm for the following purposes:

i. in treating a named partner YES☐ NO☐

Full name of partner:

ii. in treating others YES☐ NO☐

iii. in any project of research YES☐ NO☐

Please state any particular conditions as to use:

b. I hereby consent to the use of my sperm to fertilise egg(s) in vitro and to the use of embryo(s) developed from these egg(s) for the following purposes:

i. in the treatment of myself together with a named partner YES☐ NO☐

Full name of partner:

ii. in treating others YES☐ NO☐

iii. in any project of research YES☐ NO☐

Please state any other conditions as to use (eg on the use of particular embryos):

Signature: Date: DAY MONTH YEAR

II. STORAGE

a. I hereby consent to the storage of my sperm:

Storage period in years Maximum (10 years)* YES☐ NO☐ If less please state YEARS☐

*Centres are allowed to store sperm for longer periods for limited uses only.

b. I hereby consent to the storage of embryo(s) developed in vitro from egg(s) fertilised with my sperm:

Storage period in years:

Five years YES☐ NO☐ Ten years YES☐ NO☐ More than ten years YES☐ NO☐

If less than five years or some other period please state the number of years: YEARS ☐

I understand that consent to storage of more than five years must be accompanied by a completed HFEA(96)8 form which has been signed by a registered medical practitioner.**

**This does not apply to donors.

---

c. If I die or become mentally incapacitated my sperm or the embryo(s) developed in vitro from egg(s) fertilised with my sperm should:

		SPERM		EMBRYOS	
i.	be allowed to perish	YES☐	NO☐	YES☐	NO☐
ii.	continue in storage for the purpose given in 1a. (for sperm) and 1b. (for embryos) above	YES☐	NO☐	YES☐	NO☐
iii.	continue in storage for other purposes (please specify below)	YES☐	NO☐	YES☐	NO☐

_____

_____

d. Any other conditions of storage _____
(eg for particular embryos).
Please state: _____

e. I understand that unless they are used beforehand embryo(s) developed in vitro from egg(s) fertilised with my sperm will have to be allowed to perish at the end of the storage period specified at IIb.

Signature: _____ Date: DAY ☐☐ MONTH ☐☐ YEAR ☐☐

Cryostore audit

A specific email group within the clinic for patients with frozen material can be helpful in making sure that communications from patients always receive the appropriate attention.

Cryostore audit

A checklist to cover all eventualities is helpful in conducting a cryostore audit, including the question “Have the patients responded to any communication or sent any new instructions regarding consents?” If any part of the checklist for disposal cannot be completed, further investigation is required before removing any cryopreserved material from the cryostore.

Cryostore audit

Patients who have the same name: all frozen material must have a unique identifiable code, usually with **three identifying features (full name, date of birth, unique clinic accession number);** appropriate checks/witnessing will decrease the chance that cryomaterial for the wrong patients is ever removed from storage.

Cryostore audit

- A change in consent to provided extended storage
- A change in consent for removal of gametes/embryos from storage and allowing them to perish
- A couple separate, one partner requests continued storage and the other requests termination of storage. Individual gametes may be stored accordingly, but problems arise in the case of embryos. In the UK, a "cooling off" period of one year is recommended to see if the couple can agree on a united decision; if they cannot agree, storage of cryopreserved embryos must be terminated.

Cryostore audit

Couples approaching the end of their defined storage period should be contacted in advance, so that they have sufficient time to consider the options (usually to remove the gametes/embryos from storage, extend storage, or donate the material to a licensed research project).

Cryostore audit

Couples may have several gametes/embryos in storage generated in different treatment cycles. The gametes/embryos from specific dates must be used in accordance with the patients' wishes. Cryostorage from different dates should be highlighted in the patients' notes.

Cryostore audit

Mistakes can be avoided by establishing a system that includes robust checks before any gametes/embryos are removed from storage. Straw labels should have a unique cryonumber for the couple, and labels from thawed straws can be attached to the lab report for the thaw.

Cryostore audit

- Members of the embryology team change; staff should always remain up to date with all information relating to the contents of the cryostore.

Safety of Reproductive Cryopreservation

A recent meta-analysis provides new evidence about whether there are differences in terms of neonatal outcome following fresh or frozen embryo transfer cycles.

- **lower risk of prematurity or having low birthweight**
- **higher risk of high birthweights**
- **higher risk also for hypertensive disorders of pregnancy**

Safety of Reproductive Cryopreservation

For some neonatal outcomes there is no significant difference between frozen and fresh transfer strategies

- **Antepartum hemorrhage**
- **Admission to NICU**
- **Congenital abnormalities**
- **Perinatal mortality**

cryoinjuries

For successful cryopreservation, conditions should be optimized to minimize injuries and maintain a high survival rate.

The incidence of these injuries is closely related to the cryobiological properties of oocytes/embryos

- **sensitivity to chilling**
- **permeability of the plasma membrane to water and cryoprotectant agents**
- **Sensitivity to the chemical toxicity of the cryoprotectant**
- **Tolerance for osmotic swelling and shrinkage**

cryoinjuries

During cooling to subzero temperatures, cells may be exposed to several mechanisms of damage

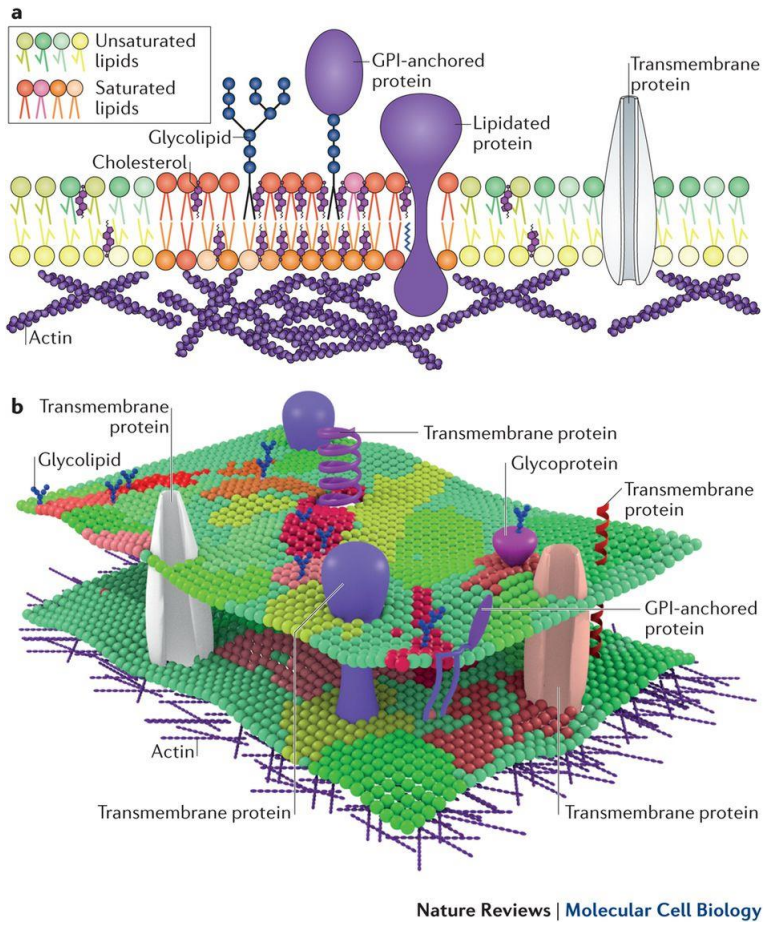
- ✓ **Chilling injury**
- ✓ **Ice crystal formation**
- ✓ **Fracture damage**
- ✓ **Multiple aster formation**
- ✓ **Osmotic stress**

Chilling injury

Chilling injury usually occurs between +15 and –5°C.
It induces partially irreversible changes in:

- ✓ **Lipid droplets**
- ✓ **Lipid- rich membranes**
- ✓ **Microtubuli of the mitotic or meiotic spindle**

Chilling injury is a common cryoinjury during application of slow freezing techniques, whereas vitrification involves a very high cooling rate and passages rapidly through this dangerous temperature zone, thus decreasing chilling injury to the oocytes and embryos.



Ice crystal formation

- ✓ It may occur between –5 and –80°C.
- ✓ Ice crystal formation is considered to be the major source of injury in the medium surrounding cells and inside the cells, including the cytoplasm and nucleus.

Vitrification:

- ➢ High concentrations of CPAs
- ➢ High cooling rates (2000–20000°C)
  Consequently, vitrification avoids ice crystal formation

Fracture damage

When cryopreserved embryos are recovered, they are occasionally found to be cracked. This physical injury, called fracture damage, is thought to be caused by non-uniform changes in the volume of the liquid and solid phases of the medium during rapid phase changes

Fracture damage occurs between –50 and –150°C, because of the mechanical effect of the solidified solution, especially in relatively large biological objects such as oocytes and embryos

Fracture damage can be reduced by reducing the cooling and warming velocities during passage through the temperature range where the phase change would occur (approximately −130°C).

Osmotic stress

During cryopreservation of cells with high osmolar cryoprotectant solutions, the cells shrink immediately as water leaves in response to the difference in osmotic pressure between intracellular and extracellular solutions

It has been reported that water leaves a cell at about 5000 times faster than that of macromolecules and other solutes present in the cytoplasm

Sucrose, is usually used as an osmotic buffer to counteract the high concentration of CPAs in the cell.

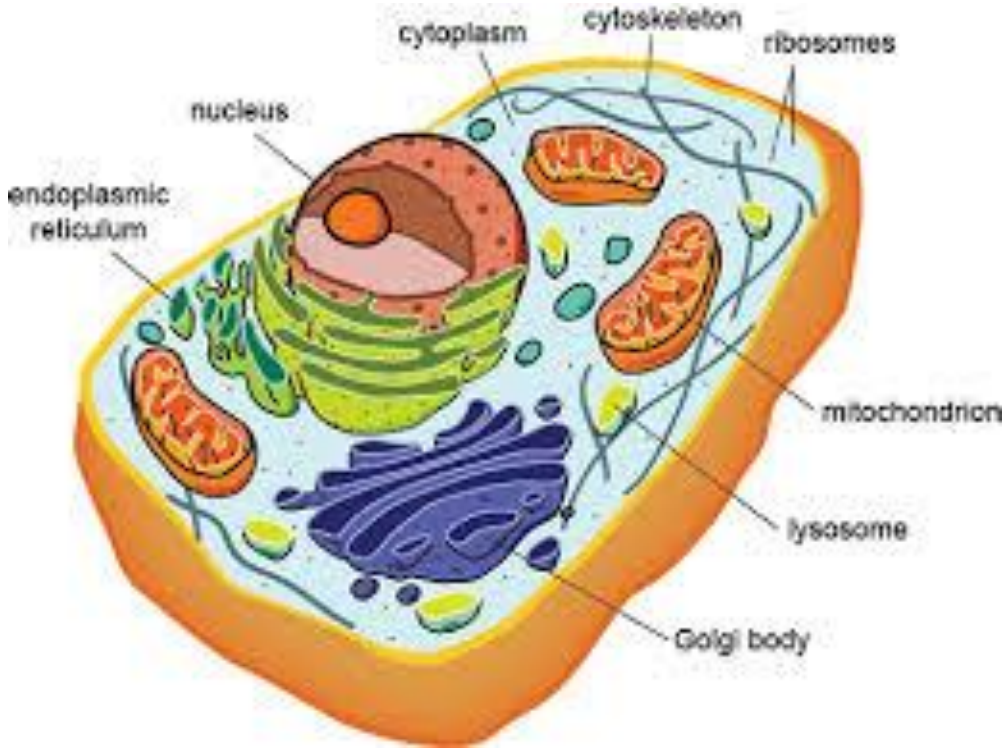
Changes in the cell shape can lead to cytoskeleton damage and fracture of the zona pellucida.

Cellular effects of cryopreservation on oocytes and embryos

Cytoskeleton

Zona pellucida

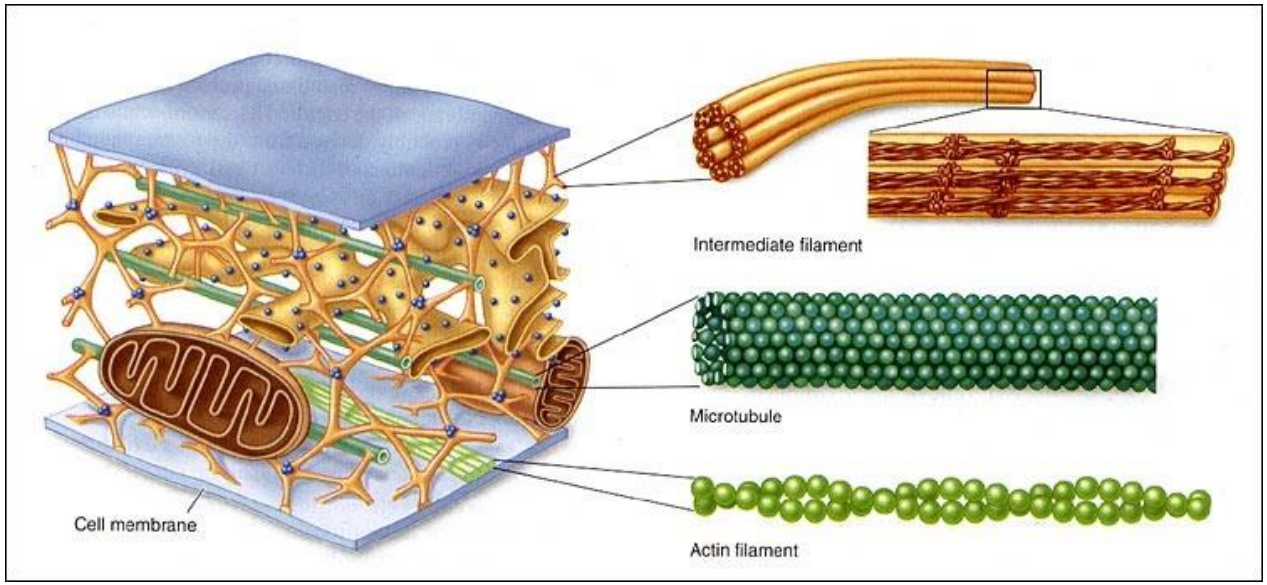
Mitochondria



Cytoskeleton

The oocyte cytoskeleton consists of three main components

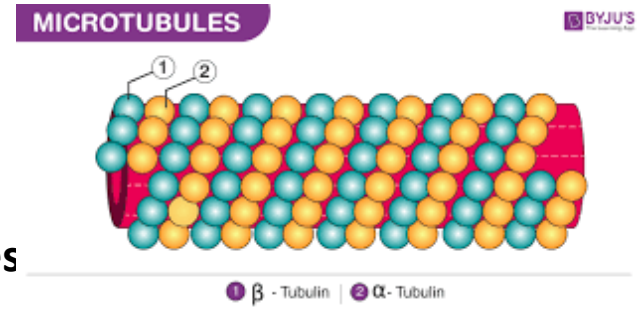
- ✓ **Microtubules (polymerized tubulin)**
- ✓ **Microfilaments (polymerized actin)**
- ✓ **Intermediate filaments**



Cytoskeleton

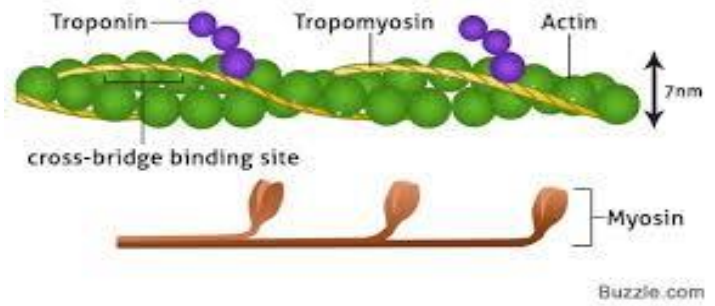
Exposure of oocytes to cooling, CPAs or the freeze/thaw process may cause microtubule depolymerization:

- **DNA fragmentation**
- **Abnormal spindle configurations**
- **Chromosomal abnormalities**
- **Altered distribution or exocytosis of cortical granules**
- **Cytoplasmic membrane fracture**



Negative influence on microfilament functions:

- **Abnormal distributions of mitochondria**
- **Reduced meiotic competence**
- **Reduced fertilizability of oocytes**
- **Failure in the preimplantation embryo**



Cytoskeleton

In oocytes, the meiotic spindles consist of microtubules that are constructed by polymerization of α- and β- tubulin. Meiotic spindles play vital roles in meiotic progression as well as chromosomal alignment and segregation

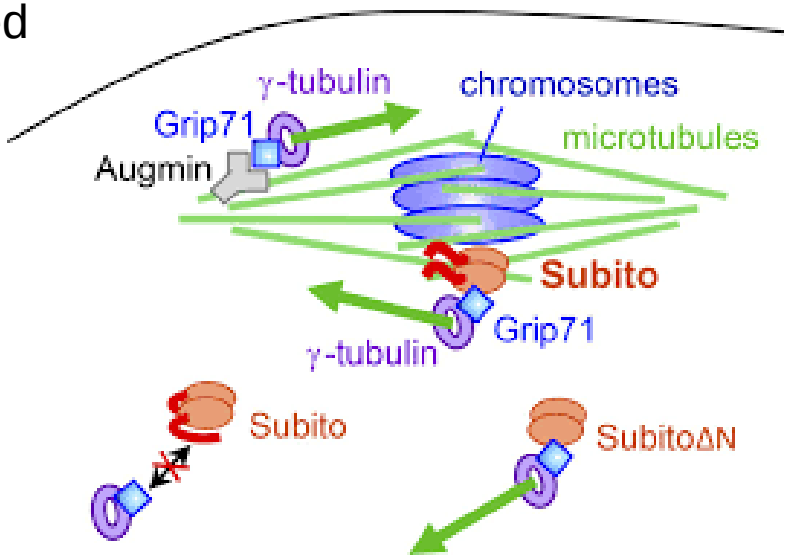
Many technological advances have enabled visualization of the spindle.

- ✓ **Confocal microscopy**

Invasive

- ✓ **Polarized light microscopy**

Non-Invasive



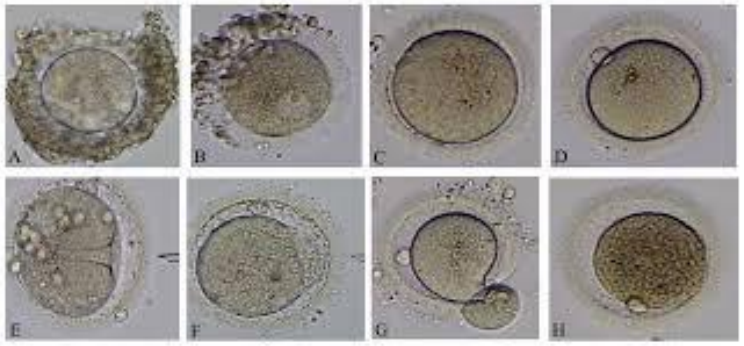
Cytoskeleton

There is disappearance and reappearance of meiotic spindles during MII after vitrification and slow freezing which depends on

- ✓ **The time interval after thawing**
- ✓ **Methods of freezing and thawing**
- ✓ **Species**

A potential strategy to avoid spindle depolymerization is cryopreservation of oocytes at the germinal vesicle (GV) stage. However, immature oocytes:

- ✓ **Less permeable to water and CPAs**
- ✓ **More sensitive to cryopreservation**
- ✓ **Live births are rarer than those achieved with mature oocytes**



Cytoskeleton

Cytokeratin is an intermediate filament that plays important roles in oocyte maturation and embryonic development .
The cytokeratin structure is affected during vitrification of both mature and immature oocytes which most likely contributes to oocyte death

Further investigation is needed to overcome the consequences of cytoskeletal injuries and enhance cryopreservation procedures.

Zona pellucida

The zona pellucida is a glycoprotein membrane surrounding the plasma membrane of oocytes and preimplantation embryos.

During cryopreservation of oocytes, CPAs cause transient calcium increases in oocytes , and thus trigger cortical granule exocytosis that is sufficient to cause zona hardening and compromises sperm penetration and fertilization

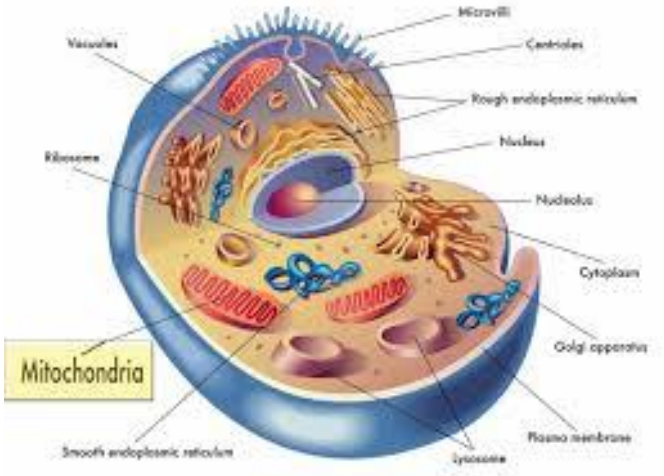
A rapid change in the cell configuration is another negative effect of cryopreservation on oocytes. Alterations of the cell shape are observed as the cell folds in on itself, forming a concave appearance and thus resulting in fracture of the zona pellucida

Mitochondria

Mitochondria are the sole source of energy production in the ooplasm to provide adenosine triphosphate (ATP) for fertilization and preimplantation embryonic development.

Vitrification has been reported to compromise mitochondrial function and reduce ATP content in human and bovine oocytes

The intracellular distribution of mitochondria is dependent on microtubules



Mitochondria

- Cryopreservation can lead to mitochondrial swelling
- Abnormally shaped mitochondria
- Rupture of their inner and outer membranes

To reduce the negative effect of vitrification on mitochondrial functions, addition of 1 mol L-glycine to vitrification solutions results in maintenance of oocyte mitochondrial function and a subsequent improvement in the blastocyst developmental rate

Thanks for attention