Oocyte & Embryo Cryopreservation

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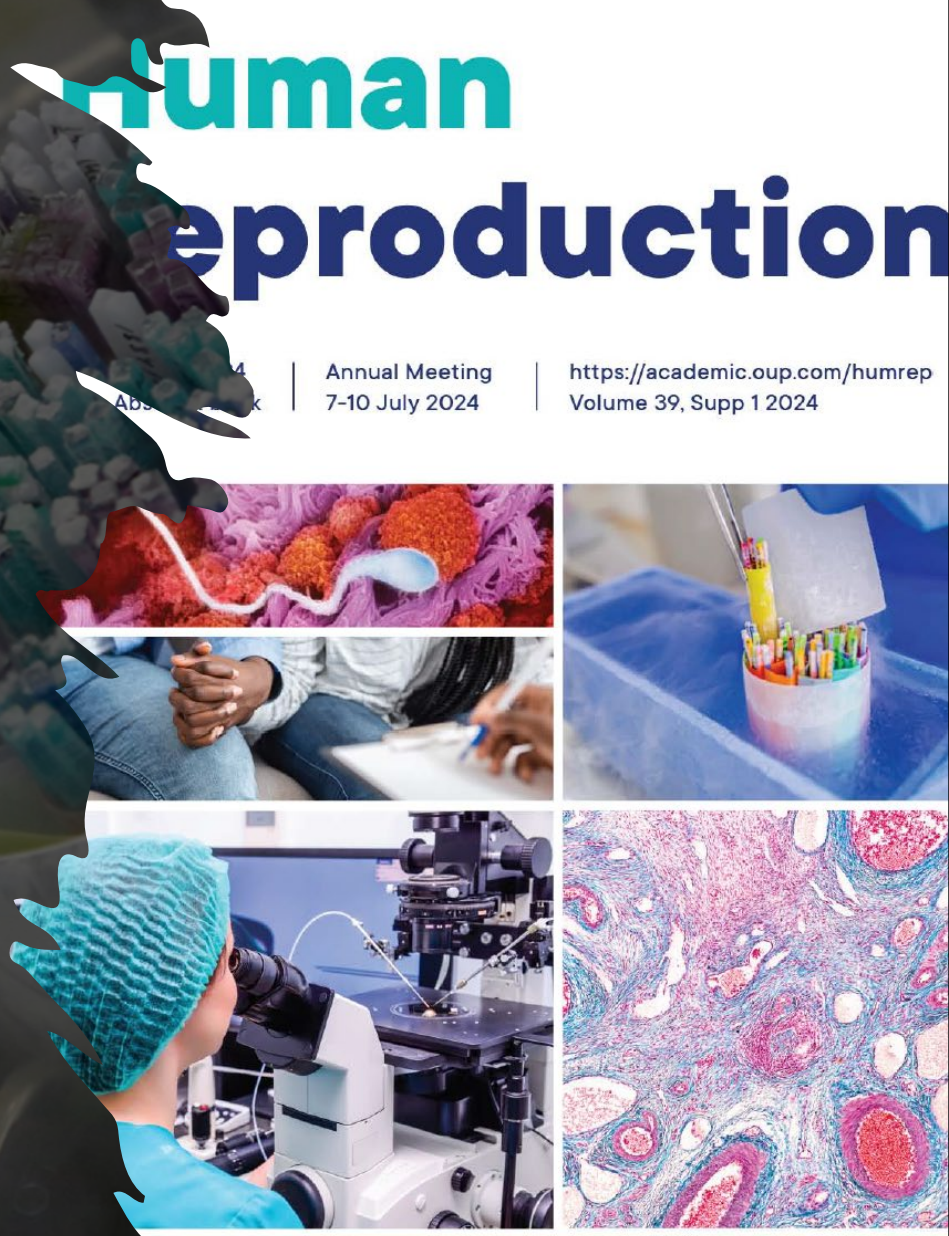

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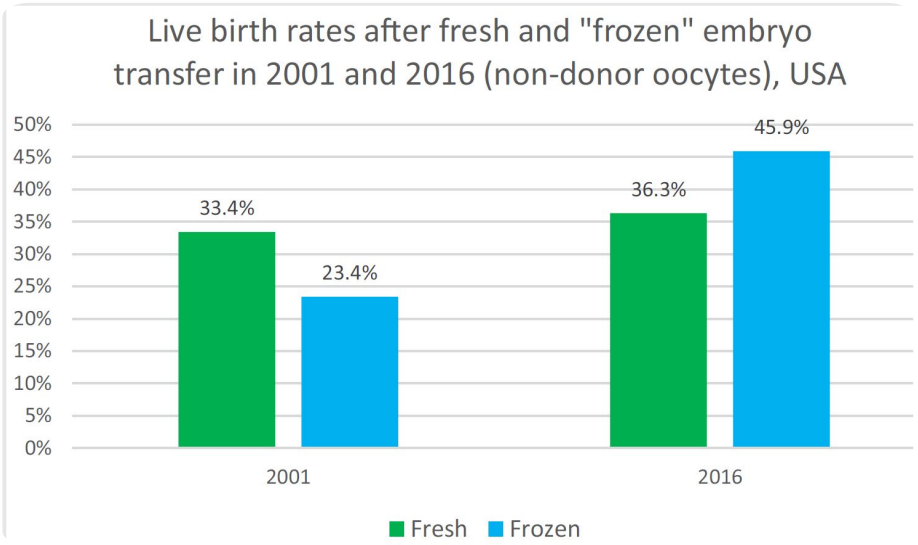
Introduction



(Zsolt Peter Nagy 2020)

Since the birth of **Louise Brown in 1978**, more than **nine million** children have been conceived using (ARTs).

1978

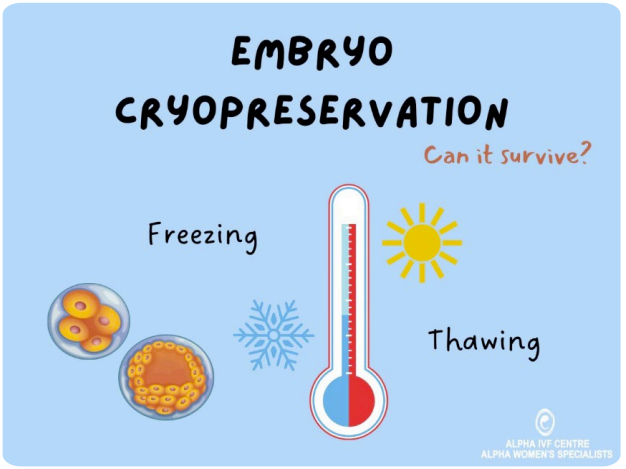
1983

The first births from frozen embryos were achieved in 1983, while the first birth from a frozen oocyte was reported 3 years later

Embryo & oocyte cryopreservation

Elective embryo cryopreservation

- for banking,
- donation,
- deferred childbearing,
- preimplantation genetic testing,
- storage of surplus embryos
- single embryo transfer,
- decreasing the risk of multiple gestation pregnancy
- hyperstimulation syndrome
- Endometrial Bleeding,
- Elevated Serum Progesterone Levels on the day of trigger (Somdeb Bhattacharya 2023),



Non-elective embryo cryopreservation

- is indicated for fertility preservation such as in patients undergoing chemotherapy (Nor Ashikin 2023).

Outcomes of female fertility preservation with cryopreservation of oocytes or embryos: a population-based study, Netherlands

- Data were collected between 2017-2019 from 1112 women who cryopreserved oocytes or embryos in the context of fertility preservation
- Fertility preservation cycles are performed increasingly over the years. In the first years (2004-2007) less than 10 cycles per year were performed, which increased to more than 300 cycles per year in ten years' time (since 2016).
- The median number of oocytes cryopreserved was 13 per woman (range 1-52) and of embryos cryopreserved was 6 (range 1-32).
- After 10 years follow-up, 25.5% of the women used their cryopreserved oocytes or embryos to attempt pregnancy and of these, 34.6% had a live birth.
- The cumulative clinical pregnancy rate after embryo transfer of cryopreserved oocytes or embryos was 39.2% and the live birth rate was 34.6% per patient.
- Fertility preservation through freezing oocytes or embryos is an established treatment for women with a risk of **premature ovarian failure**

Acceptance rates and reasons for social oocyte cryopreservation among women: systematic review and metaanalysis. Turkey

- Oocyte cryopreservation is used for different purposes such as fertility preservation, social reasons and assisted reproductive treatments.
- the overall acceptance rate of social oocyte cryopreservation was found to be 54% .
- Acceptance rates when the cost of SOC is covered was 57% and this rate was 49 % in the absence of a partner .
- Findings of this review underline that affordable oocyte cryopreservation will increase the use of SOC among women of reproductive age.

Oocyte cryopreservation: a comprehensive analysis of live birth rate by age and indication of treatment provides reassurance for young cancer patients, Melbourne, Australia

- Women undergoing autologous IVF treatment using vitrified and warmed oocytes. Outcome of stimulation cycles and warmed oocyte cycles were assessed according to women's age at OCP (35 years, 35-40 years and 40 years) and treatment indication- cancer diagnosis, other medical conditions and non medical.
- no differences in rate of cycles with at least one good quality embryo rates (cancer diagnosis 87.2%, other medical 82.1% and non medical 89.3%, p¼0.06).
- patients with cancer had lower oocyte survival rate (80.8% vs 84.8% in other medical and 82.1% in non medical, p<0.01). Patients with cancer diagnosis had higher clinical miscarriage rate (25%) compared with other medical (7%) and non medical (10.5%), p<0.01.

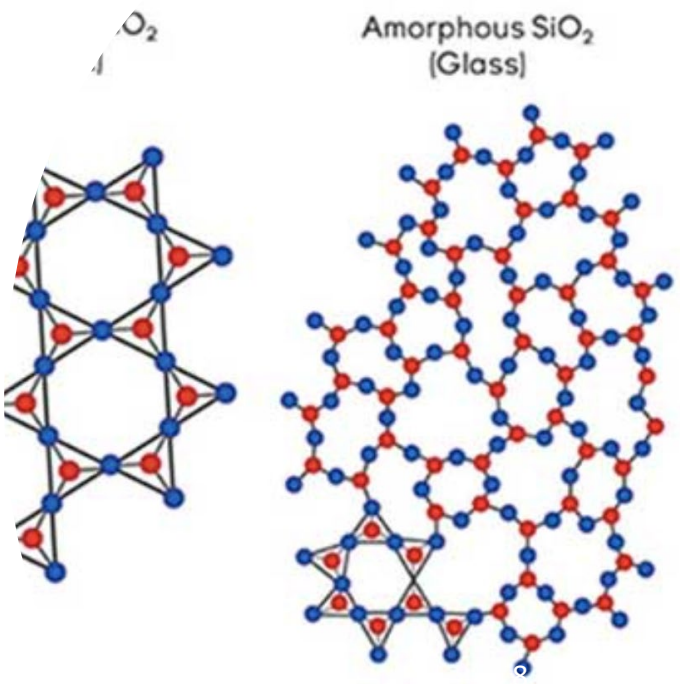
Advances in oocyte freezing: implications for donors, recipients, counsellors and clinics,United Kingdom

- Much of the attention given to the introduction of oocyte vitrification has focused on the implications for autologous freezing – i.e. freezing by those who wish to store their own eggs for later use.
- Whilst use of frozen eggs for donation varies by country and clinic, data show that in general the practice is growing; with Spain an early and enthusiastic adopter within Europe.
- The advent of egg vitrification has led to or accelerated a number of changes in the landscape of donation. More efficient and reliable freezing allows clinics to 'batch' eggs and store them for potential use by multiple recipients thereby increasing clinic revenue.

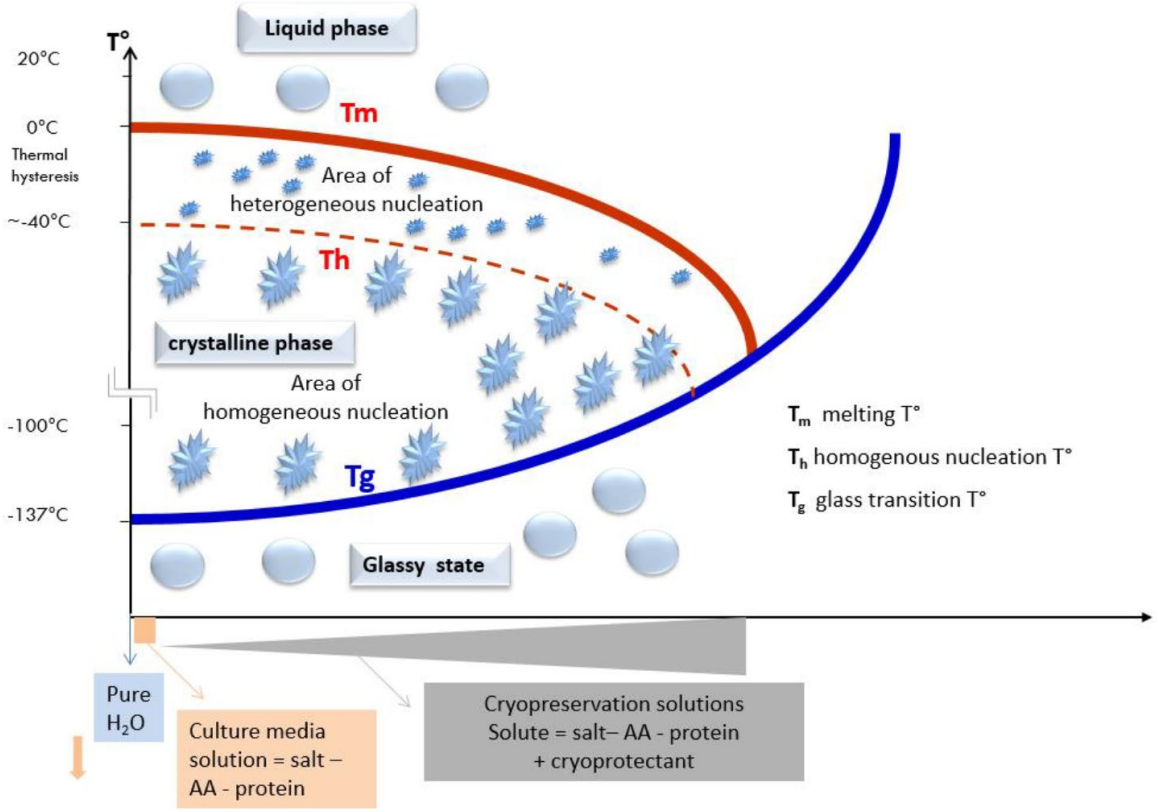
- **Factors that determine cell survival from cryopreservation**
- the osmotic response
- CPA have a great capacity to form hydrogen bonds with water molecules, due to their hydroxyl residues (glycerol, ethylene glycol, 1-2 propanediol, propylene glycol) or sulfoxide groups (e.g., DMSO). Pomeroy et al. 2022
- dissemination over the lipid bilayer, which is exceptionally temperature-dependent and hydrophilic temperature-independent channels made by proteins called aquaporins (Somdeb Bhattacarye 2023).
- Cryoprotectant (Permeable CPAs, Impermeable CPAs)

-Permeable CPAs : include substances such as 1,2- propanedial (PROH), dimethylsulfoxide (DMSO), ethylene glycol (EG), and glycerol.

-Impermeable CPAs: Low molecular weight disaccharides such as sucrose and trehalose



In solutions, the content of salts, proteins, and other macromolecules, as well as cryoprotective agents (CPA), lead to an increase in viscosity.



CPAs increase the viscosity and thereby lead to a slowdown of the molecular movements of the water. An increase in viscosity results in **(i) a delay of the nucleation phenomenon, (ii) a reduction of the growth rate of ice crystals, (iii) a limitation of the size of the crystals between Tm and Tg, and (iv) an impairment of crystal formation in the case of the huge increase in viscosity during drop in the T.**

Consequently, the melting temperature I decreases, and the glass transition temperature (Tg) increases. Thereby, the temperature range where ice crystal formation occurs becomes substantially smaller, and the probability of obtaining a glassy state increases. Vaderzwalmen et al. 2020

1. Oocyte vitrification

- used what became known as the Cryotop method with 15% DMSO and 15% ethylene glycol and 0.5 M of sucrose.
- During these days, both oocytes and embryos are routinely vitrified using these methods, and few improvements have been made to these techniques since 2003.

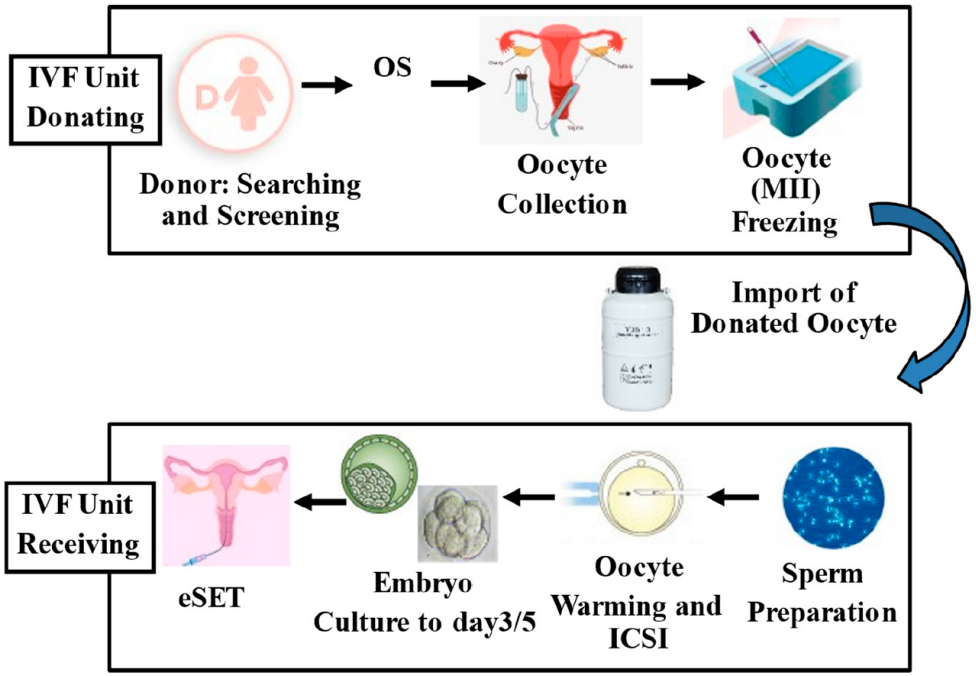
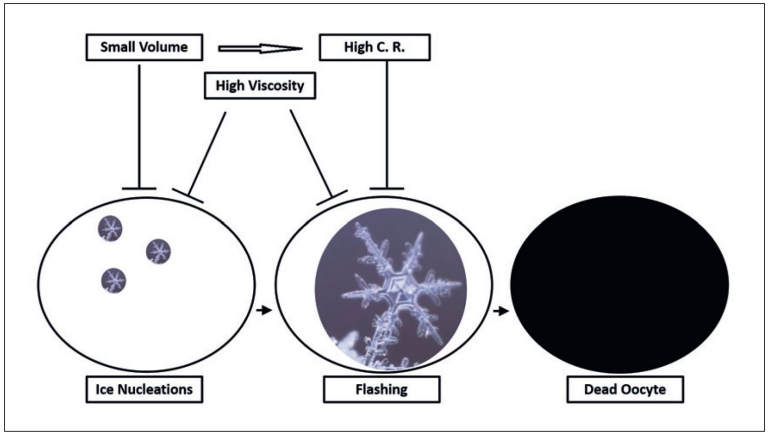


Figure 1. Description of the imported oocyte donation program from a foreign oocyte bank. eSET, elective single embryo transfer; ICSI, intracytoplasmic sperm injection; MII, metaphase II oocyte; OS, ovarian stimulation.



Review Article

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The Near Future of Vitrification of Oocytes and Embryos: Looking into Past Experience and Planning into the Future

Amir Arav Yehudit Natan

Fertilesafe Ltd., Nes-Ziona, Israel

- Oocytes were discovered as more difficult to cryopreserve than embryos.
- Changes in oocyte membrane fatty acid composition by either nutrition or fusion with liposomes affected biophysical parameters and chilling sensitivity causing increased chilling sensitivity to oocytes.
- the viscosity is the most important factor since it affects both the chances for ice nucleation to occur as well as the ability to progress afterwards.
- The sample's volume influences the chances of ice nucleations to form as well as the cooling rate, and the cooling rate affects the ability of the crystals to progress
- Mazur [1990] found that if ice crystals occupy 16% of the oocyte they will cause cell death.

Time of Oocyte Denudation Before Vitrification

- Oocytes usually are completely denuded before freezing to assess their maturation.
- similar survival rates whether oocytes were denuded or not (Minasi MG 2012, Tong XH, et al. 2012).
- vitrified cumulus-free oocytes undergo a faster recovery of the meiotic spindle than vitrified partially denuded ones (Minasi MG 2012) and 2 hours after warming are assumed to be required to let the spindle repolymerize and to recover the plasticity of their membranes during the puncture by the ICSI needle.
- Oocytes can be vitrified between one and six hours after ovum pick-up, and immediately after denudation.
- however, the redox homeostasis appears shifted toward an oxidated state after vitrification, suggesting the putative need for antioxidants added to vitrification/warming solutions and culture media to improve embryo development (Nohales-Corcoles M, 2016).

Ten years' experience using cryopreserved oocytes from In Vitro Maturation (IVM) or Controlled Ovarian Hyperstimulation (COH) cycles for fertility preservation in oncologic indications, France

- Fertility Preservation (FP) techniques are proposed to patients with cancer to provide the possibility of childbearing using their own gametes.
- Currently, oocyte vitrification after COH is the standard option. IVM serves as an alternative when COH is contra-indicated, urgent cancer therapy is required, or in conjunction with ovarian cortex cryopreservation.
- Seventy-seven warming attempts followed by ICSI respectively from 37 COH and 40 IVM cycles were analyzed.
- Oocyte survival, and fertilization rates and clinical outcomes were similar in COH and IVM groups,
- We reported a significantly better embryo quality at day-2 in the COH group when compared to the IVM group,
- Limited live births have been reported from frozen-thawed oocytes in IVM cycles of cancer survivors.
-

It's worth the freeze: outcomes from thawing electively cryopreserved oocytes are comparable in patients with reduced vs normal ovarian reserve at the time of cryopreservation, Boston, USA

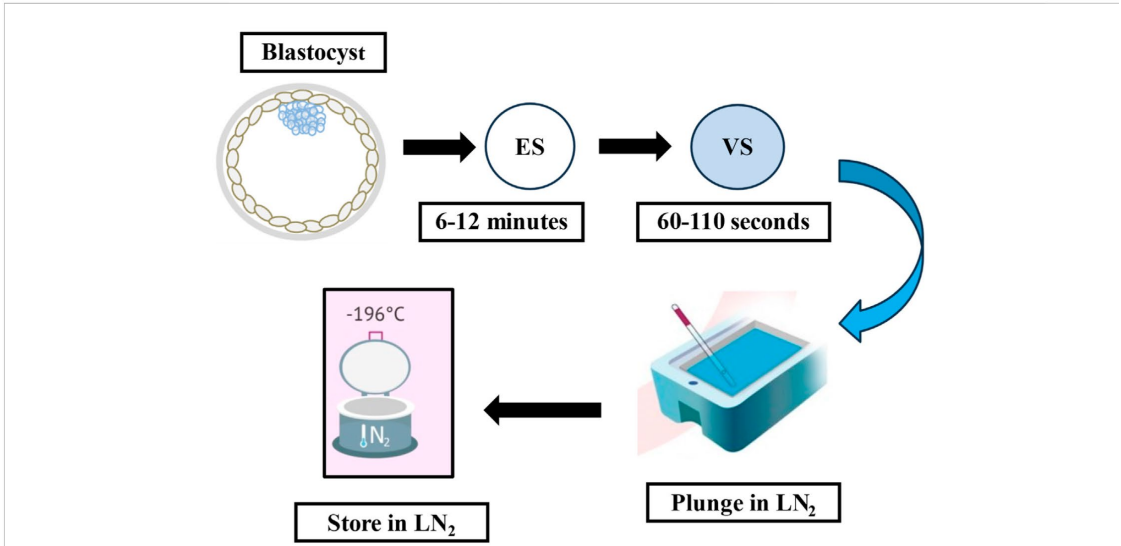
- AMH categories of<1, 1-2 and > 2 were selected to stratify reduced, low-normal, and normal ovarian reserves, respectively.
- Average oocyte yield per cryopreservation cycle was significantly different across AMH groups (7.7 oocytes in AMH < 1, 10.5 oocytes in AMH 1-2, and 17.2 oocytes in AMH > 2, p<0.01).
- Oocyte survival (mean 89.0%), fertilization rate (mean 75.3%), and blastocyst conversion rate (mean 62.4%) did not vary significantly across AMH groups.
- The rate of PGT-A euploid embryos did not vary between groups (38.8% in AMH < 1, 45.3% in AMH 1-2, 53.0% in AMH > 2, p¼0.07).
- Clinical pregnancy, miscarriage rate, and live birth rate in the first transfer cycle was not impacted by AMH.

Egg vitrification related to female age: survival and laboratory outcome of 1233 vitrified-thawed oocytes in nonmedical autologous use, France

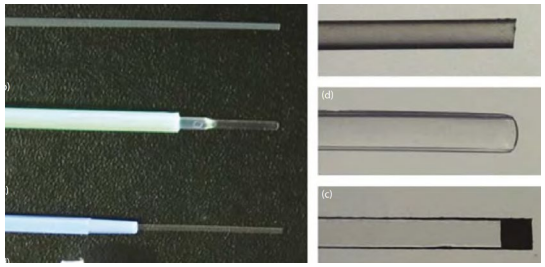
- Retrospective study on 166 patients with frozen- thawed oocytes who underwent ICSI during 2021-2023 in a single hospital. Patients were divided into three groups according to their age at retrieval (Group A37 n¼77, Group B 37-40 n¼44, Group C40 n¼45).
- 1043 eggs were injected and the post-ICSI lysis rate was 11.2%.
- No significant differences were seen in thawing survival rate in groups A, B and C (85.6% vs. 83.5% vs. 83.8%, p¼0.635) nor in the post-ICSI lysis rate (13.1% vs. 7.4% vs. 11.2%, p¼0.072).
- 2PN fertilization rate was similar between the groups (67.8% vs. 61.3% vs. 67.7%, p¼0.178)
- triploid fertilization rate (3PN) statistically grew with age (2.8% vs. 5.8% vs. 6.5%, p¼0.034).
- In patients over 40, thawed oocytes display more abnormal fertilization, develop less in extended culture and yield less top quality blastocyst.
- oocyte freezing at a younger age is recommended,

2. Embryo vitrification

1. the equilibration was performed in equilibration solution for **10 min** in Cryotop method, at room temperature.
2. Afterwards, the embryos were placed in vitrification solution for 50–60 s
3. Then, the embryos (maximum 3 in each vitrification device) were immediately aspirated with a minimum volume of the vitrification solution and placed onto the tip of Cryotop® vitrification devices.
4. CPA toxicity is its most significant disadvantage. on the other hand, High concentrations of CPA are necessary to prevent intracellular ice crystals during vitrification.



(Kuwayama et al., 2005).



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ORIGINAL ARTICLE

Reproductive Medicine and Biology
WILEY

Effect of equilibration time on clinical and neonatal outcomes in human blastocysts vitrification

Shingo Mitsuhata | Momoko Hayashi | Yoshitaka Fujii | Hiroaki Motoyama |

- This is a retrospective study based on data collected between November 2008 and November 2015. A total of 192 blastocysts (80 non-expanded and 112 expanded) obtained from 167 patients were analyzed.
- The blastocysts were divided into two groups according to their equilibration time: 8-11 minutes or 12-15 minutes.
- The survival, implantation, and live birth rates of non-expanded blastocysts were not different between the two groups, but they significantly improved for the expanded blastocysts in the 12-15 minutes group compared to the 8-11 minutes group.
- For the non-expanded blastocysts, a shortened equilibration time (8-11 minutes) is sufficient for effective vitrification.

TABLE 3 Clinical outcomes of non-expanded and expanded blastocysts following 8-11 and 12-15 minutes' equilibration vitrification protocols

	Non-expanded blastocysts			Expanded blastocysts		
Warming cycle results	8-11 min	12-15 min	95% CI	8-11 min	12-15 min	95% CI
Warming cycles	40	40	-	52	60	-
Type of endometrial preparation						
Natural cycles (per warming cycles)	31 (77.5%)	28 (70.0%)	0.55-3.95	40 (76.9%)	50 (83.3%)	0.27-1.67
HRT cycles (per warming cycles)	9 (22.5%)	12 (30.0%)	0.25-1.82	12 (23.1%)	10 (16.7%)	0.60-3.76
Warmed blastocysts	40	40	-	52	60	-
Blastocyst morphology[a]						
Good (per warmed blastocysts)	13 (32.5%)	11 (27.5%)	0.49-3.27	36 (69.2%)	43 (71.7%)	0.40-2.00
Fair (per warmed blastocysts)	24 (60.0%)	22 (55.0%)	0.51-2.96	15 (28.8%)	15 (25.0%)	0.53-2.78
Poor (per warmed blastocysts)	3 (7.5%)	7 (17.5%)	0.10-1.49	1 (1.9%)	2 (3.3%)	0.07-4.50
Survived blastocysts (per warmed blastocysts)	39 (97.5%)	38 (95.0%)	0.26-16.2	46 (88.5%)*	59 (98.3%)*	1.16-50.0
Implantation[b] (per warmed blastocysts)	8 (20.0%)	8 (20.0%)	0.34-2.91	12 (23.1%)**	27 (45.0%)**	1.21-6.14
Live birth (per warmed blastocysts)	5 (12.5%)	7 (17.5%)	0.20-2.23	8 (15.4%)***	23 (38.3%)***	1.39-8.31

Note: Values presented as number.

Abbreviations: CI, confidence interval; HRT, hormone replacement therapy.

Prolonged exposure to equilibration solutions may be detrimental to an embryo's developmental potential, whereas a shorter exposure may affect the penetration of cryoprotectants into blastomeres.

Relationship between exposure in equilibration solution pre-vitrification for human collapsed blastocysts and miscarriage rate in ART treatments, Switzerland

- prospective study was performed in China from March2018 to May 2022.
- A total of 831 vitrified warmed blastocysts were analysed in this study, of which 825 survived at the warming step (99.3%: 825/831).
- ES time: time of 7-8 minutes compared to the group of 9- 10 minutes.
- same survival rate after warming for group A (99.3%) and group B (99.3%), as well as similar CPR (A: 69.1% versus B: 68.0%).The live birth (A: 63.8% versus B: 58.3%) and multiple gestation rates (A: 20.8% versus B: 23.5%) were comparable in the two groups.
- When analysing the overall miscarriage rate, data displayed a statistically significant difference ($P<0.05$) in favour of group A (7.6%) compared to group B (14.2%).
- Results demonstrate that a shorter equilibration time resulted in optimal survival, clinical pregnancy, and live birth rates compared to exposure to ES for 9-10. Thus, suggesting that a longer exposure to the ES is not needed, and might negatively effect cellular metabolism and function, increasing the risk of miscarriage.

Warming from -190 to +20°C

- The risk of injury during warm-up is the same as during cool-down.
- Rapid thawing can protect cells by preventing small intracellular ice crystals from recrystallizing into larger, more dangerous ice crystals.
- Impermeable CPA, which is lost after thawing, creates an osmotic gradient that controls the passage of water across cell membranes and helps avoid osmotic shock.
- Because cryoprotectant are usually less permeable than water, removal of cryoprotectants during warming must be performed gradually to prevent cell bursting (Renard JP, 1983).

✓ the 1-min one-step rehydration approach has shown consistently high survival rates and, more importantly, comparable implantation and pregnancy rates (Manne et al., 2021; Manns et al., 2022; Taylor et al., 2022; Liebermann et al., 2023 (Liebermann et al., 2023)Sciorio et al. 2024

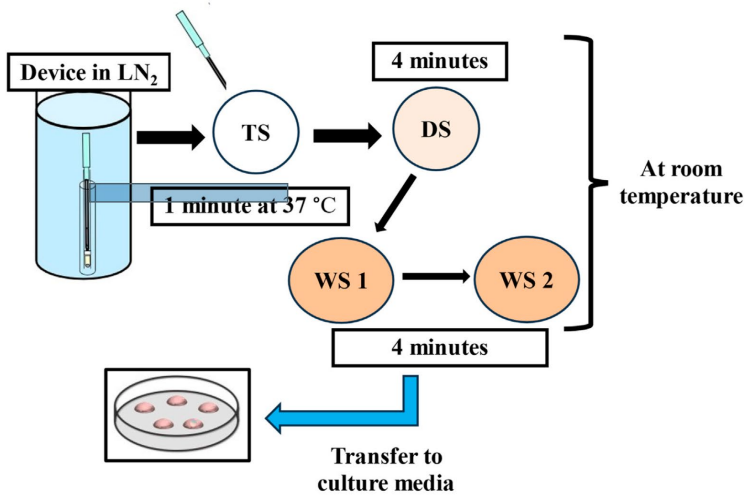

FIGURE 2
The flow-diagram shows the warming procedure for vitrified blastocysts. TS: thawing solution, DS: dilution solution, WS: washing solution, LN_2: iquid nitrogen.

Ultra-fast warming of blastocysts yields better survival rates for D5 non-PGT and similar survival rates for D5/D6 PGT blastocysts, Belgium

- Blastocysts, were ultrafast warmed by immersing the straw in a 37 C thawing solution (1M trehalose; Vit-kitwarmNX; Fujifilm) for 1 minute.
- Ultra-fast warmed blastocysts showed a total re-expansion rate of 93.3% in 3.3h § 2.7h on average.
- Ultra-fast warming, shows significantly better rates of survival and post-warming development in vitro for non-PGT blastocysts and similar rates for PGT blastocysts.
- Minimizing handling steps leads to an overall risk reduction in potential exposure to suboptimal warming conditions and operator/handling-related stress. This optimization brings huge efficiency to the busy work schedule that is characteristic for an IVF lab without dropping cryopreservation quality indicators.

Elevating human oocyte vitrification and warming to a new level: fast, efficient, and effective, USA

- Oocyte vitrification and warming can be done successfully in 4 minutes, reducing the time of commonly used current protocols by up to 25 minutes.
- From November 2022 to November 2023, 504 GV and 207 MI oocytes, which did not convert into mature oocytes at day of oocyte retrieval, were vitrified in Vit Kit Freeze (FujiFilm Irvine Scientific, USA), and RapidVit Oocyte (Vitrolife, Sweden).
- A group of 4 GV or MI oocytes was vitrified at room temperature for 1 minute in Equilibration solution (ES) and 1 minute in Vitrification solution (VS), loaded on a S-Cryolock (Biotech, USA)
- Warming was done at 37C in 0.5M sucrose for 1 minute and Wash solution (WS) for another minute.
- Warming in 0.5M instead of 1M sucrose contributes positively to survival and allows earlier resumption of functionality.
- there was no statistically significant difference in the survival rate of GV and MI oocytes between Irvine and Vitrolife media and was comparable to fresh oocytes.

Human oocyte survival, early embryo development, metabolic fingerprinting, and pregnancy outcomes following ultrarapid or standard vitrification and thawing, Slovakia

This prospective study included 1,214 donor oocytes

There was 100% survival in oocytes subject to ultra-rapid vitrification-thawing (n¼531) compared to 94.5% in oocytes that underwent standard vitrification.

There were no significant differences in the fertilization rates following intracytoplasmic sperm injection (81.3% with ultra-rapid and 83.2% with standard vitrification).

Ultra-rapid vitrification thawing produced significantly more blastocysts but both techniques had similar euploidy rates (61.8% vs. 61.4%, respectively; $p>0.05$).

clinical pregnancy rates were 65.22% with ultrarapid and 56.5% [RL2] with standard vitrification.

Fast vitrification and warming protocols demonstrate similar efficiencies to a standard method and a substantial reduction in execution times. Spain

Standard volumes, solutions and devices (Cryotop) were used as indicated by the manufacturer for standard protocols (Kitazato).

Experimental groups included a control with standard vitrification/warming (Kitazato), standard vitrification with fast warming (1min in TS), and fast vitrification (1min in ES and 1min in VS) with fast warming (1min in TS).

Survival rates after vitrification and warming were statistically similar between groups.

Fast vitrification and warming protocols demonstrated efficiency rates similar to a standard protocol in all pre-clinical models tested and a significant reduction in execution times.

Comparison of clinical outcomes between overnight warmed and embryo transfer day warmed blastocysts in frozen embryo transfer cycles, Singapore

- Some studies reported that the resumption of mitosis in thawed cleavage-stage embryos resulted in significantly better clinical outcomes in FET cycles.
- Unnecessary transfer of non-functioning embryos can be avoided by incubating thawed embryos overnight
- Cryopreserved blastocysts were routinely warmed one day before embryo transfer (ET) However, when ETs fell on Monday or post public holiday, vitrified blastocysts were warmed on ET day (ETDW group).
- Overall, clinical pregnancy rates (CPR) and live birth rates (LBR) were significantly higher in the ETDW group (CPR: 47.47% vs. 40.47%, $P<0.01$; LBR: 34.99% vs. 28.78%, $P<0.05$).
- CPR (45.02% vs. 25.71%, $P<0.0001$) and LBR (32.03% vs. 17.45%, $P<0.0001$) were significantly increased when vitrified day 6 blastocysts were warmed and transferred on the actual day of ET.
- Warming of a vitrified blastocyst **on ET day is preferred regardless of blastocyst age.**

Keshavarzi et al. Middle East Fertil Soc J (2021) 26:24
https://doi.org/10.1186/s43043-021-00068-1

2021

Middle East Fertility Society Journal

RESEARCH Open Access

Post-warming survival rates and clinical outcomes of human cleavage stage embryos vitrified/warmed using CryoTouch and Cryotop methods

Somayeh Keshavarzi[1], Azadeh Dokht Eftekhari[2], Hajar Vahabzadeh[2], Marzieh Mehrafza[2], Robabeh Taheripanah[1,3], Masoumeh Asgharnia[4], Sahar Esfandyari[1,5], Alaleh Ghazifard[1], Hossein Hosseinirad[1*] and Shahrokh Paktinat[1*]

- This retrospective study was conducted to analyze the survival rate of cleavage stage embryos after vitrification/ warming performed by CryoTouch and Cryotop methods, and the subsequent clinical outcomes in two infertility clinics located at Erfan Niayesh Hospital Tehran, Iran and Mehr Fertility Research Center, Rasht, Iran, from January 2018 to December 2020.
- CryoTouch method (RS Medical, Ravan Sazeh Co., Tehran, Iran) or Cryotop method (Kitazato BioPharma Co., Shizuoka, Japan).
- This retrospective study evaluated a total of 173 FET cycles performed on 446 warmed cleavage stage embryos.
- The results showed no significant differences between two groups in terms of post-warming survival rate (p value = 0.5020), clinical pregnancy rate (p value = 0.7411), implantation rate (p value = 0.4694), and live birth rate (p value = 0.5737).

Methods

- CryoTouch, the equilibration was performed in equilibration solution for **7 min** in CryoTouch method or **10 min** in Cryotop method, both at room temperature.
- Afterwards, the embryos were placed in vitrification solution for 50–60 s in both protocols.
- Then, the embryos (maximum 3 in each vitrification device) were immediately aspirated with a minimum volume of the vitrification solution and placed onto the tip of CryoTouch® or Cryotop® vitrification devices.

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OPINION

Are commercial warming kits interchangeable for vitrified human blastocysts? Further evidence for the adoption of a Universal Warming protocol

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- This is a longitudinal cohort study analysing two hundred fifty-five blastocysts warming cycles performed between January and October 2018.
- Embryos were vitrified using only one brand of ready-to-use kits (Kitazato), whereas the warming procedure was performed with Kitazato, Sage and Irvine.

✓ Kitazato vitrification and warming solutions contain trehalose and are supplemented with hydroxypropyl cellulose (HPC);

✓ Sage contains sucrose with human serum albumin (HSA);

✓ Irvine kits contain sucrose with dextran serum supplement (DSS).

Comparative study between two commercial vitrification kits on survival and clinical outcome for blastocyst frozen embryo transfer, Malaysia

- Recent study suggested that cell apoptosis in specimen vitrified in trehalose media is lower in comparison to sucrose.
- The overall mean post thawed survival was higher in Kitazato (95.8%) compare to Irvine (91.8%) ($p<0.001$).
- the Irvine group shown to have significantly higher clinical pregnancy
- the implantation rate (IR) in Irvine group group (55.7%) is also significantly higher compare to the Kitazato group (50.5%).

immediately post-thaw from the 2 h post-thaw diameter.

- Group 1 included embryos that continued **to shrink by 10 µm or more after the 2 h** measurement;
- group 2, embryos with measurement differences ranging **from 9 to 9 µm** according to the 2 h measurement;
- group 3 included those demonstrating a **re-expansion trend of 10 m or more.**

Journal of *Clinical Medicine* 2022 MDPI

Article

Standardization of Post-Vitrification Human Blastocyst Expansion as a Tool for Implantation Prediction

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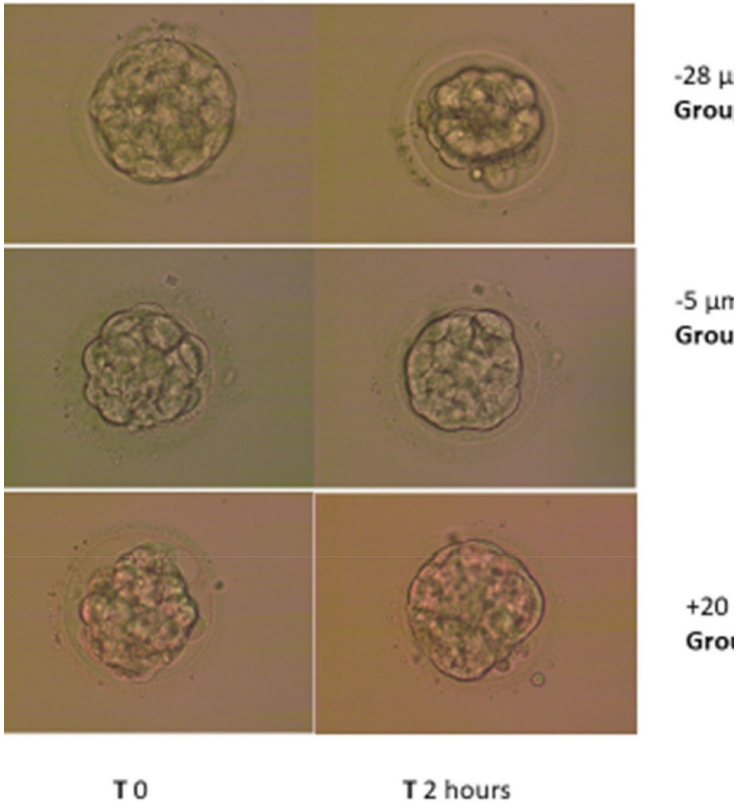


ure 2. Examples of embryonic measurements and grouping criteria.

Freezing and thawing were performed using the **SAGE kit** (Cooper Surgical, Trumbull, CT, USA), according to the manufacturer's recommendation. The freezing device used was either **a Cryotop (Kitazato, Japan) or Cryolock (Biotec, Alpharetta, GA, USA).**

Table 2. Main demographic, clinical and clinical pregnancy data according to study groups.

Study Group* (*n*)	Age (at Oocyte Retrieval)	N Oocytes Aspirated	Endometrial Lining, mm	Fertilization Method (%)		Clinical Pregnancy
				IVF	ICSI	
1 (37)	33.65 ± 6.01	12.38 ± 6.08	8.6 ± 2.1	54.1	45.9	7 (18.9%)
2 (37)	32.89 ± 5.04	13.89 ± 7.12	9.4 ± 1.9	67.6	32.4	10 (27.0%)
3 (41)	33.63 ± 6.07	14.22 ± 8.31	9.1 ± 1.5	57.9	42.1	21 (51.2%)
p-value	0.88	0.50	0.16	0.49		0.007

Numbers represent mean ± SD or *n* (%); * Group 1—Shrinking ≥10 µm; group 2—Change between −9 and +9 to µm; group 3—re-expansion trend ≥10 µm.

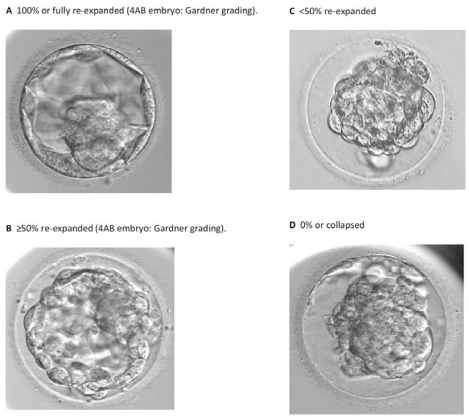
Table 3. Clinical pregnancy rate according to age at oocyte retrieval.

Study Group *	Age < 35 Years	Age ≥ 35 Years
	Clinical Pregnancy *n* (%)	Clinical Pregnancy *n* (%)
Group 1	20 (25)	17 (11.8)
Group 2	23 (34.8)	14 (14.3)
Group 3	21 (52.4)	20 (50.0)
p-value	0.07	0.009

* Group 1—Shrunk ≥10 µm; group 2—Change between −9 and +9 µm; group 3—re-expansion trend ≥10 µm.

Conclusions:

- blastocysts with a higher developmental stage demonstrate higher level of glucose uptake [Ferrick L,2020]. Therefore, blastocyst re-expansion may reflect cell viability.



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EMBRYO BIOLOGY

Check for updates

Post-warming embryo morphology is associated with live birth: a cohort study of single vitrified-warmed blastocyst transfer cycles

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- Purpose This study aims to examine whether blastocyst morphology post-warming correlates with live birth.
- performed between November 2016 and May 2017.
- Immediately before transfer, the degree of blastocoel re-expansion was graded as:
- A, fully expanded; B, partially expanded ≥ 50%; C, partially expanded < 50%; and D, collapsed.
- The degree of post-warming cell **survival** was graded on a scale of 50 to 100% and was then classified into 4 groups: very low 50–70%, low 71–80%, moderate 81–90%, and high 91–100%.
- Human Reproduction and Embryology (ESHRE) report on ART laboratory performance indicators selected the degree of re-expansion as the best post-thaw parameter for the prediction of live birth [ESHRE,2017].
- blastocysts with poor post-warming morphology still demonstrate a considerable probability of live birth.

Time Between Biopsy and Vitrification

- Still debated is whether the timing of vitrification after biopsy impacts on cryo-survival rates and clinical outcomes after warming.
- Three studies reported on this topic, 2 suggesting a trend toward better outcomes per transfer if blastocysts were vitrified within 30–60 minutes from biopsy (Xiong S 2021 - Maggiulli R 2019), and one claiming the opposite, namely that blastocysts vitrified >180 minutes after biopsy are subject to higher full reexpansion and improved clinical outcomes after warming (Chen HH 2017).

Can embryo re-expansion pattern post-biopsy for PGT-A have a predictive value on the outcome?United Arab Emirates

- Retrospective study of 1141 single euploid blastocyst transfers (seFET) conducted between April 2021 and February 2023.
- Blastocysts were scored at a standardized time (1hr post TE-biopsy): 0 for complete collapse, 1 for starting re-expansion, and 2 for clear re-expanded cavity.
- Some studies support prolonged culture post-biopsy (> 3hrs), citing improved implantation and pregnancy rates. Conversely, others advocate for rapid cryopreservation, endorsing an immediate approach within an hour.
- One-hour post-biopsy 64.0% of blastocysts re-expanded to Grade 2, 29.3% to Grade 1, and 6.7% showed no re-expansion (Grade 0).
- Regression analysis showed reexpanding to Grade 2 at 1-hour post-biopsy was significantly and independently associated with OPR/LB compared to embryos that did not re-expand

Post-warming morphokinetics as a predictive tool for embryo implantation, Brazil

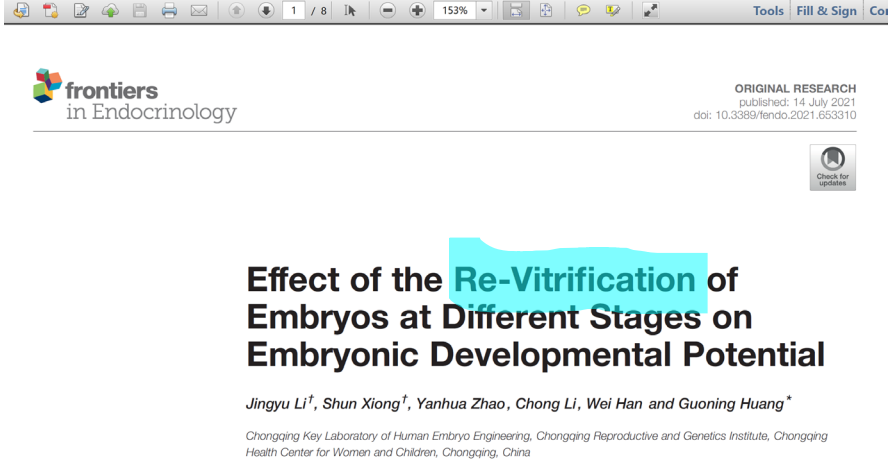
- Throughout vitrification and warming processes, embryos face considerable challenges.
- post-warming culture in TLI incubators may provide a more precise assessment of vitrified and warmed blastocyst behavior.
- included 411 vitrified/warmed blastocysts,
- After warming, the recorded factors were: (i) initial blastocyst area (IBA); (ii) maximum blastocyst area (MBA); (iii) Blastocyst area delta (DBA); (iv) initial ZP thickness (IZP); (v) minimum ZP thickness (MZP); (v) ZP thickness delta (delta ZP area); and (iv) blastocyst expansion (BE).
- Embryos received a final score from 0 to 9, according to the sum of the scores from each parameter and were grouped as: Low total-score, medium total-score), and high total-score.
- A significant increase in the implantation rate was noted among embryos presenting higher scores,

Double vitrification

- Double vitrification, using a vitrified egg to create an embryo that then undergoes a successful freeze/ thaw cycle, was first reported in 2008 (Chang CC, 2008) and is now a commonplace occurrence with the advent and growth of frozen donor egg banks (Nagy ZP 2009).

- The outcomes from these embryos are excellent (survival rate, implantation and pregnancy rates remain comparable to those of "fresh" embryos) (Cobo A, 2013).

- Wang's 2023. investigation reported that double vitrification is associated with no significant difference was found in neonatal outcomes.



frontiers in Endocrinology

ORIGINAL RESEARCH
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Check for updates

Effect of the Re-Vitrification of Embryos at Different Stages on Embryonic Developmental Potential

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Retrospective and mouse experimental studies.

- fresh (group 1),
- vitrified at the 8-cell stage (group 2),
- vitrified at the early blastocyst stage (group 3),
- vitrified at the 8-cell stage, and re-vitrified at the 8-cell (group 4) or
- early blastocyst stage (group 5).

- These results showed that re-vitrification at the blastocyst stage had a notable negative effect on embryonic developmental potential, while re-vitrification at the 8- cell stage did not.

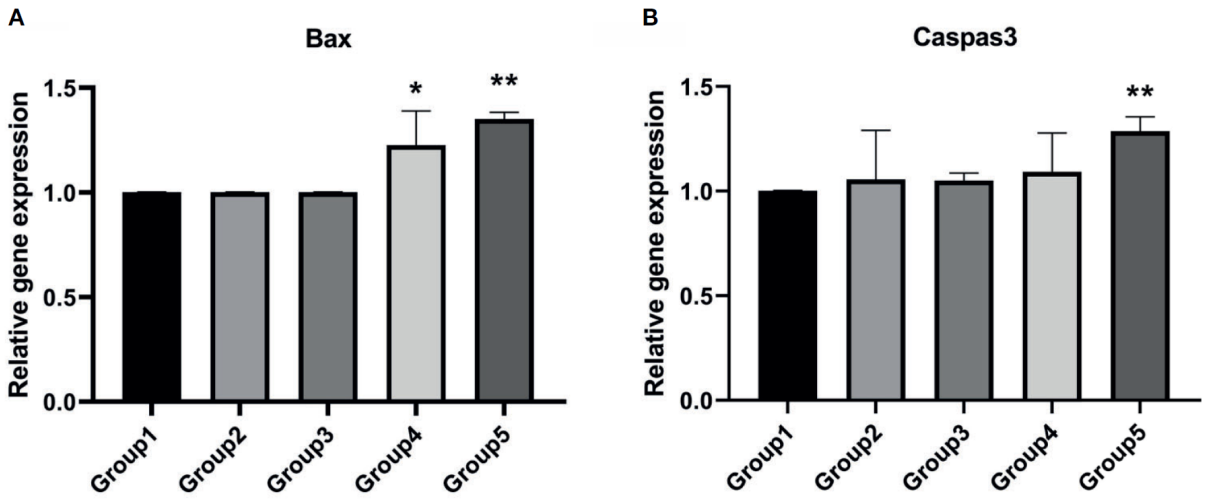


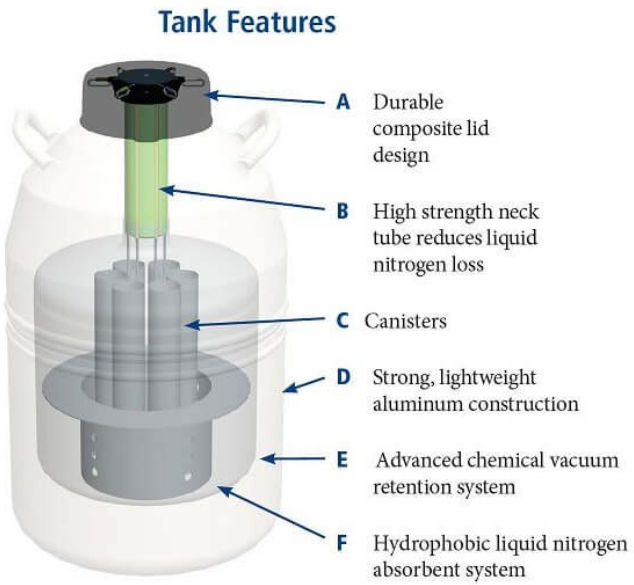
FIGURE 3 | Expression levels of apoptotic genes. **(A)** Group 4 and group 5 showed a significantly higher expression level of *Bax*. Values are shown as the mean ± the standard error of the mean (SEM). **(B)** Group 5 showed a significant higher expression level of *Caspase3*. Significant difference, *P < 0.05, **P < 0.01.

- Therefore, since recryopreservation might impair embryo viability, clinical teams should maintain a cautious attitude

Transferring blastocysts derived from frozen-thawed cleavage embryos versus frozen-thawed blastocysts: A comparative analysis of pregnancy outcomes, Vietnam

- Regarding the D3-5 group, a noteworthy proportion of 65.3% cycles were unfortunately cancelled.
- the blastulation rate in the D3-5 group (40.1%) was lower than in the D5 group (46.4%),
- We propose that the use of frozen-thawed blastocysts may be a more efficient and patient-friendly option.

- Evaluating the impact of embryos undergoing multiple freeze-thaw cycles in preimplantation genetic testing for aneuploidy (PGT-A) on treatment outcomes in IVF, United Kingdom
- Transfer of twice-thawed euploid embryos demonstrated comparable clinical pregnancy outcomes, yet elevated miscarriage rates following a repeated vitrification-warming cycle warrants further scientific inquiry.



Cryo-survival duration

- a recent retrospective study reported no differences in embryo cryo-survival, pregnancy, and neonatal outcomes in relation to cryo-storage duration up to 7 years (Li X, Guo P, 2023).
- 18 years.(Yuanlin Ma 2021) (Zsolt Peter Nagy 2020).
- ≥34.81 months(Cobo et al., 2024).

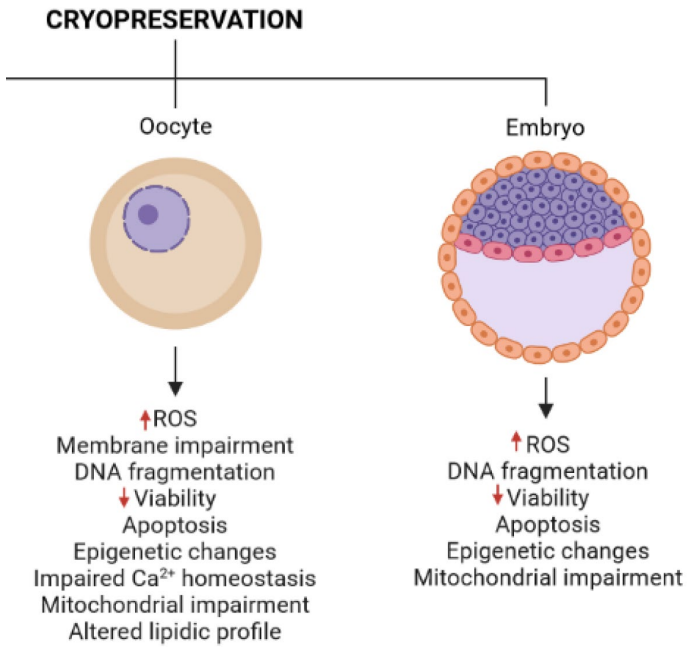
interval between the start of embryo cryopreservation and frozen/thawed embryo transfer does not influence pregnancy outcomes.

Embryo long-term storage does not affect ART outcome: analysis of 58,001 vitrified blastocysts, Spain

- This was a retrospective multicenter study including a total of 58001 vitrified/warmed day-5 blastocysts elective FET which involved patients undergoing freeze all cycles and non-elective FET including 41,386 embryos from 25,571 patients.
- Embryo survival did not show statistical differences across the categories of storage time in freeze-all and non-elective FET groups.
- Long-term storage does not affect live birth rate (LBR) in elective and non-elective FET cycles.

molecular changes by cryoprotectant in embryo:

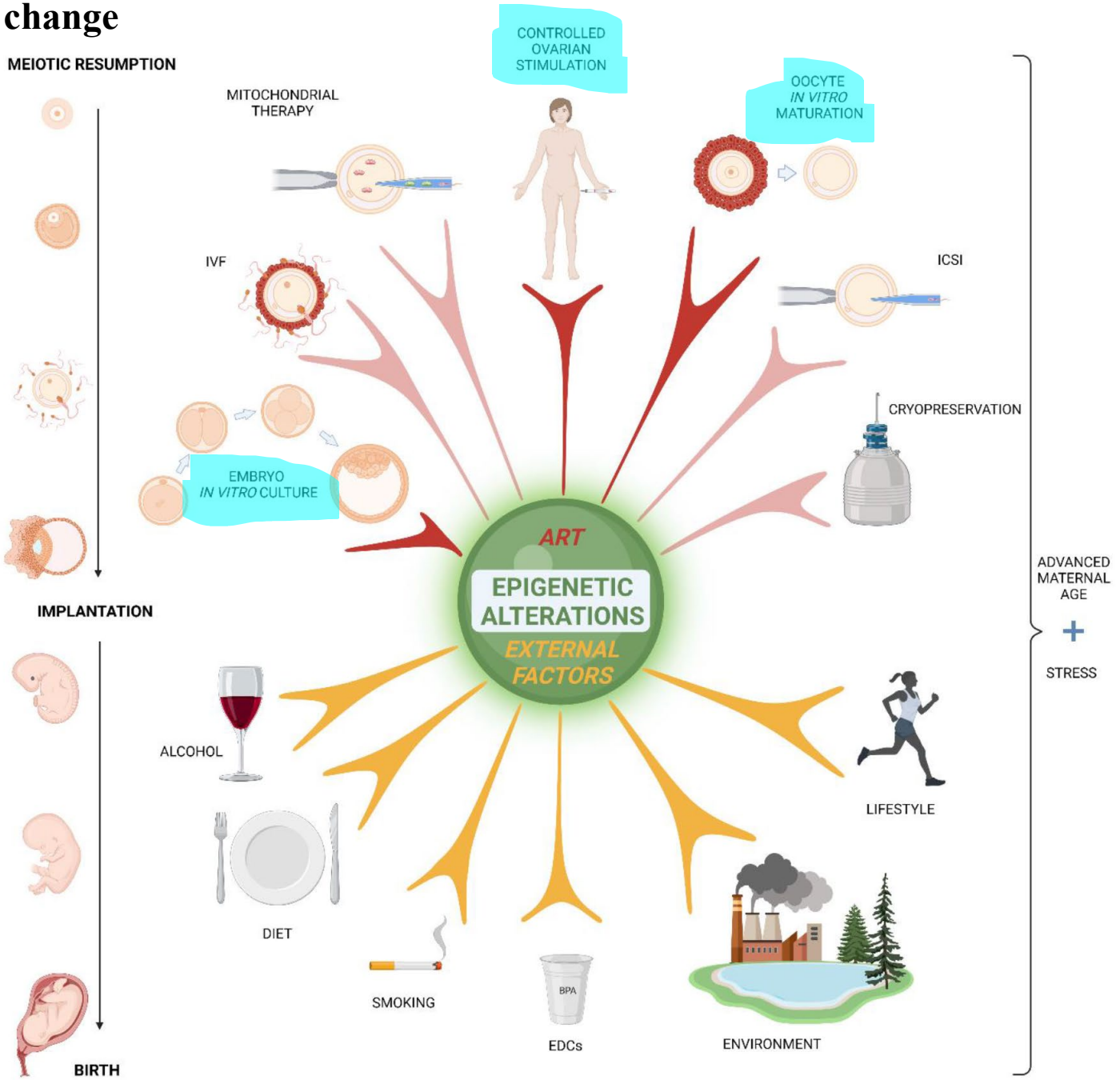
- affecting molecular impairments related to metabolism,
- cytoarchitecture,
- calcium homeostasis,
- epigenetic state
- DNA methylation
- Reduce ATP content
- cell survival
- DNA integrity,
- cell death,
- hardening the cell membrane
- alter mitochondrial distribution,
- induces deregulation of the proteomic profile ,
- neurodegenerative diseases. Estudillo et al. 2021

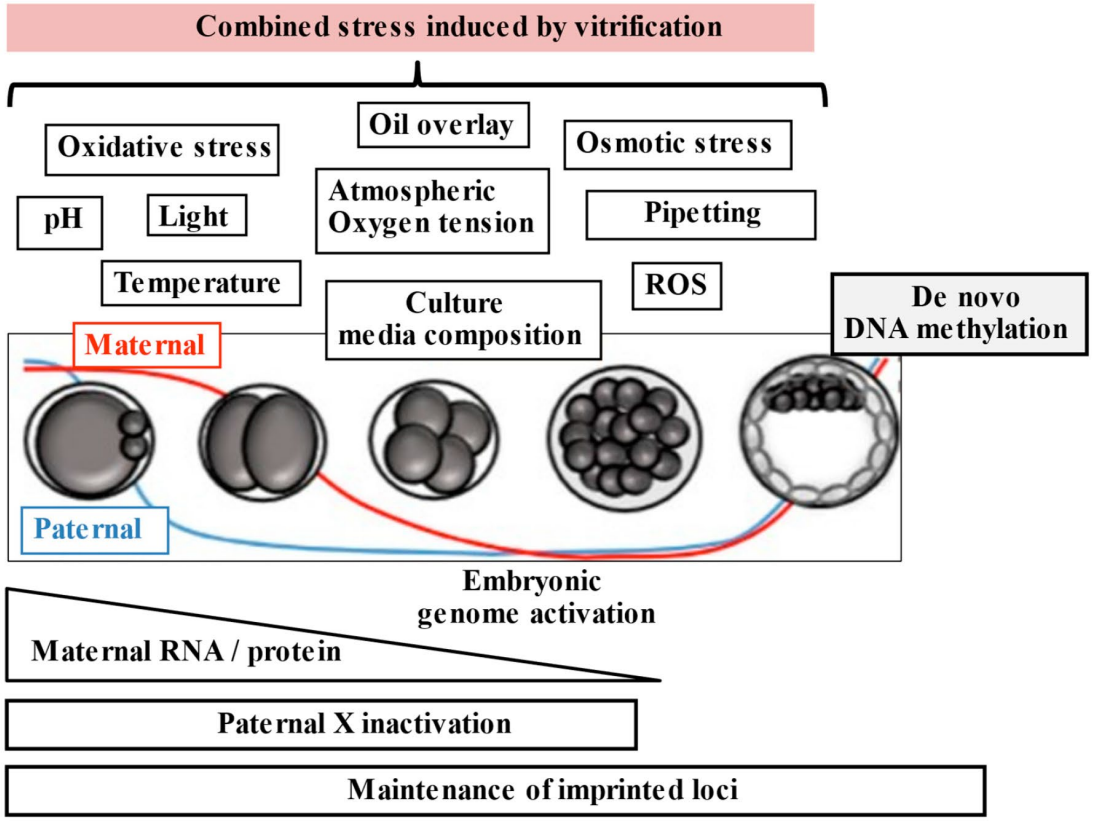


children born following vitrification:

- increased risk of placental problems,
- pregnancy induced hypertension,
- pre-eclampsia following FET
- caesarean section, and
- preterm delivery, (Sazonova et al., 2012; Opdahl et al., 2015).
- high birthweight, LGA mortality . (Rosalik et al., 2021)
- higher risk of childhood cancer(epithelial tumors and melanoma after any ART method, and of leukemia after FET. Sargisian et al, 2022 .)

D. Epigenetic change





There are two epigenetic reprogramming phases:

1. resets DNA methylation marks in primordial germ cells (PGCs) when they migrate to the fetal gonadal ridge.
2. wave of DNA methylation changes occurs during the early stage of embryo development, following fertilization; the parental genome is actively demethylated, while the maternal genome is passively demethylated with a wave of re-methylation at the blastocyst stage

Vitrification induces a deviation in cleavage morphokinetic patterns and transcriptomic signatures along mouse embryo development, Sweden

- Recent population-based register studies have reported: 1) an increased risk of infant mortality ; 2) high birth weights; 3) an increased risk of obstetrical complications in singletons conceived using cryopreserved embryos; 4) a higher incidence of childhood cancer
- notable delays in the developmental rhythm of cryopreserved embryos compared to fresh embryos ($p<0.01$).
- immunofluorescence staining of nuclear 5mC and DNMT1 in 8-cell embryos and blastocysts demonstrated similar levels between fresh and cryopreserved embryos. global DNA methylation remained unaffected by cryopreservation.


Thanks for your attention