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Post Thawing Survival Rate Of Human Embryos In Slow Freezing Versus Vitrification: A Narrative Review

Dr. Aishwarya Grace Unnikrishnan, Dr. Navneeth Ranjith, Dr. Afifa Khaleelullah, Dr. Madiha Bano Rehmani, Dr. Arjun Jay Prakash, Dr. Nazneen Naveed Sait, and Dr. Maryam Baqri

Department of Gynecology
Ivane Javakhishvili Tbilisi State University Tbilisi, Georgia
Email: aishwaryaputhusseri@gmail.com

Abstract – The study compared the outcomes of slow-freeze and vitrification methods for oocyte cryopreservation, analyzing five randomized controlled trials, two cohort studies, and eight systematic reviews and meta-analyses. Slow-freeze is performed at room temperature using a buffered medium supplemented with gentamicin and human serum albumin. It contains 1.5 M 1,2-propanediol and 0.1 M sucrose. On the other hand, vitrification has been the preferred method since 2007. During vitrification, embryos are initially incubated in a solution consisting of 7.5% ethylene glycol and 7.5% dimethyl sulfide, both in Ham's F-10 media. This solution is further supplemented with 20% Albuminal-5. After the initial recovery, the oocytes are aspirated and immersed in a vitrification solution composed of Ham's F-10 medium for a duration of 50 to 60 seconds. The cooling is done via liquid nitrogen, and embryos are stored for months. The survival rate of embryos is the percentage of those that survive after being warmed up. Live birth rates are calculated as the percentage of live births per transferred embryo and warmed embryo. Embryos are selected by biopsy at the zygote or blastocyst stage using non-invasive methods to optimize the success rates of in vitro fertilization. Vitrification has a greater success rate, better survival rates, and transferable embryos, it is chosen for oocyte cryopreservation. Better clinical pregnancy rates and implantation capacity have also been linked to it, notably for blastocysts from In Vitro Maturation programs. To assess effects on neonatal outcomes and congenital abnormalities, additional study is necessary.

Keywords – Assisted human reproduction, Egg freezing, Cryosurvival, Infertility, Embryo survival assessment, vitrification, slow freezing, Cryoprotectant, Assisted reproductive technology, Post-thaw Implantation.

I. INTRODUCTION

Egg freezing, or oocyte cryopreservation, is a procedure used to maintain reproductive potential in women by removing, freezing, and conserving their eggs. This technique is a crucial component of assisted reproductive technology (ART) that is being utilized increasingly, particularly with cancer patients. Nevertheless, oocyte cryopreservation has been disregarded because of its vulnerability to physical and chemical stress, resulting in poor success rates. Conventional cryopreservation procedures can harm cells owing to the creation of ice and excessive solute concentrations but in vitrification processes high doses of cryoprotectants are employed to prevent it. The pace of introduction and removal of cryoprotectants as well as temperature optimizations are crucial since these high concentrations might be detrimental to cells. Quick freezing and reheating can reduce the amount of cryoprotectants that are required. Human gametes, embryos, and gonads may all be preserved using vitrification procedures ([1],[2],[3],[4]).

To maximize the safety and efficacy of In Vitro Fertilization (IVF) and to preserve fertility, there must be effective cryopreservation of oocytes and embryos. Cryopreservation uses vitrification & slow freezing methods. Results from randomized trials and cohort studies demonstrate that vitrification is better than slow freezing concerning clinical outcomes for oocytes and cryosurvival rates, cleavage-stage embryos, and blastocysts. This research shows that laboratories must consider moving from slow freezing to vitrification for cryopreservation. Yet, there are contradictory viewpoints on the success rates of open and closed

Corresponding Author: Dr. Aishwarya Grace Unnikrishnan

vitrification. Improving cryopreservation techniques and thawing/warming procedures is required to enhance the survival of follicles in cryopreserved oocytes ([1],[2],[5]). Vitrification is a superior method for preserving oocytes compared to slow freezing, as it yields similar IVF results to fresh oocytes without any additional perinatal or obstetric morbidity. To maximize live birth rates and ensure financial viability, elective oocyte cryopreservation between the ages of 35 and 37 is recommended. However, the efficacy of oocyte preservation relies on the mastery of other associated ARTs such as In Vitro Maturation (IVM), IVF , and Embryo Tranfer(ET). Currently, vitrification is the preferred method for preserving embryos and oocytes ([6],[7],[8]).

Recent developments in vitrification technology have increased oocyte cryopreservation's success, leading to higher rates of live birth and pregnancy. But there is still room for betterment in techniques for vitrification and challenges remain in developing less toxic vitrification solutions and safety devices. Thawing & mature oocyte vitrification are still considered experimental techniques due to insufficient solid data. The first attempt of Oocyte cryopreservation was using slow freezing techniques, but these procedures had low success and pregnancy rates. Technology of vitrification allows temporary quarantine of donor eggs for testing of transmissible diseases. Egg-banking programs for female fertility preservation are expected to be established worldwide with the advancements in oocyte vitrification technology, despite the ongoing struggle to achieve its goals [9].

II. Materials And Methods

Case-control studies, randomized control trials and cohort studies that were well designed are included in the study and their outcomes were analysed to find the better method out of slow-freeze and vitrification of oocytes from women. Only fully published and peer reviewed articles were included. Data collected was to assess if vitrification increases the chance of survival of oocytes as compared to slow freeze cryopreservation.

The search for relevant human studies was performed using search terms: 'oocyte', 'vitrification', 'slow freezing', 'birth', 'fertilization', and 'survival' The references are taken only if they contained these terminologies [10].

A. Study identification

The search for studies yielded studies that could potentially get the answer to the research question and further evaluate the study based on full-text articles that compared post-thawing embryo survival rates after slow freezing and vitrification among which 5 were clinical trials/RCTs ([3],[4],[5],[6],[7]) 2 where cohort studies and 8 systematic reviews and meta-analysis ([10],[11],[12],[13],[14],[15],[16],[17],[18]).

B. Slow-freezing method

The slow freezing method is done at room temperature. Embryos are placed for 10 minutes in a homemade freezing solution with buffer medium which contains gentamicin and human serum albumin 0.5% supplemented with 1,2-propanediol 1.5 M and sucrose 0.1 M. They are then loaded and placed in a freezer machine with programmed cooling technology for long preservation. For thawing the oocytes, they are plunged in water baths for 5 seconds at 30 °C and released in thawing solution and transferred to a dish containing micro drops of cleavage or blast medium [13].

C. Vitrification method

Vitrification has been the preferred method since 2007 for cryopreservation. Embryos are incubated first in solution in equilibrium containing EG 7.5% and DMSO 7.5% supplemented with abluminal -5 in Hams F-10 media for 5 to 15 minutes at room temperature. After the shrinkage and initial recovery of embryos, they are placed and aspirated in the vitrification solution that is the Samas e Ham's F-10 media for 50-60 seconds. After that cooling is done via liquid nitrogen and are stored for months. The day they are removed for thawing and warming, embryos are exposed a to thawing solution and then transferred to a dilution solution of sucrose 0.5 M for 3 minutes, then transferred to G-1™ [5].

D. Embryo survival assessment

Before the transfer of embryos, the day before they are warmed and checked if they have up to 50% surviving cells but if they have 100% surviving cells, they are considered fully intact. The live birth rates are also calculated by the percentage of births live every warmed and transferred embryo [6].

III. Discussion

Evaluation And Selection Of Embryo

IVF procedures allow for the selection of embryos using biopsy, either at the zygote stage or the blastocyst stage. Much effort and money are spent creating innovative, non-invasive methods for choosing the best embryo for transfer to improve the procedure. The techniques used in this research are metabolic profile, amino acid profile, respiration rate, and birefringence imaging of the embryo. Metabolic profiling measures metabolic alterations in the culture media of embryos using spectrophotometric assays. Proteins produced by the embryo released into the culture medium are identified using proteomic profiling. The culture medium is utilized in amino acid profiling to assess the embryo's synthesis and depletion of amino acids. Birefringence imaging makes use of polarization light microscopy to analyze the meiotic spindle of the zona pellucida, while respiration-rate measurement measures the respiration rate of the embryos. The study of various selection techniques is driven by the notion that choosing embryos is essential to enhance IVF success. This is because following thawing, cryopreserved embryos have a decreased likelihood of implanting. It is anticipated that improving selection methods would boost live birth rates while reducing the number of multiple pregnancies [19].

Following the embryo's selection, all the embryos and oocytes were grown in the same medium that was covered in mineral oil. Both traditional IVF and ICSI employing frozen testicular sperm or ejaculated sperm were used to fertilize the oocytes. Based on the number and size of the blastomeres (whether they were equal or unequal, i.e., with a difference in size greater than 25%), and the degree of fragmentation (ranging from 0% to over 50%), assessments of the embryo development were made on day 2 (41–44 hours after insemination) and 3 (66–71 hours) and, the embryo was transferred at the end of Day 3 [20].

For cryopreservation, the embryos were divided into three groups. The Vitrolife method and media were used for vitrification in one group, the Irvine methodology and media were used for vitrification in another group, and the slow-freezing group experienced a slow-freezing procedure using the proper medium. The Vitrification High-Security packages and tubes from CryoBioSystem were utilized for storage for the vitrification and slow-freezing groups, accordingly. During the warming cycles, patients undergoing embryo transfers were given either hormone therapy cycles using estradiol valerate or natural cycle preparation. Micronized progesterone was given in doses of 2 mg for the natural cycle and 3 mg for the hormone cycle to help with the luteal phase [11].

Using a programmed freezer, the embryos were gently frozen using a single-step freezing medium of cryoprotectant that contained 1,2-propanediol and 0.1 milligrams of Sucrose. The embryos were put into secure straws one by one following a 10-minute incubation period. The cooling rate for the freezing program was 2°C/min down to 5°C, and it remained for ten minutes, hand seeded, and then kept for an additional 10 minutes at 5°C. The freezing program started at 20°C. The rate of freezing then decreased to 0.3°C/minutes until 30°C, before increasing to 50°C/minute until -150°C. After that, the straws were placed in a container filled with liquid nitrogen for storage. The straws were immersed in a 37°C water bath for 30 seconds after being submerged in a three-step solution beginning with 0.25 M sucrose for defrosting. The straws had been placed in a 37°C water bath after 30 seconds of air exposure as part of a three-step thawing procedure that started with a solution of 0.25 M sucrose. The fertilized eggs were placed in a total of three medium for five minutes each after the straw had been cut, and the thawing procedure was conducted at room temperature [21].

A process known as vitrification turns a liquid that has been cooled to an incredibly low temperature into a solid that resembles glass while preventing the growth of ice crystals. This method works by combining a high concentration of cryoprotective agents (CPAs) with a very quick cooling rate. However, excessive CPA levels can endanger the cells. Thus, to achieve the required concentration for vitrification, vitrification procedures typically use two or more CPAs, while minimizing their toxic effects with a low concentration of each. Any penetrating CPA can be used for vitrification but owing to its quick diffusion into cells and minimal toxicity, EG is seen to be the best option. Because smaller samples need less liquid to be cooled and are less likely to form ice crystals, size has a significant impact on vitrification. Low liquid volumes can be attained using a variety of techniques, such as medium drops, hard surfaces, silver-sealed vitrifying systems, and straws of plastic [22].

The embryos were kept alive using a process called vitrification and a cryoprotectant solution, each embryo was vitrified separately. The embryos were transferred to four successive droplets of vitrification solution (containing 15% DMSO, 15% EG, and 0.5 milligrams of sucrose in a HEPES-buffered medium) for a total of five seconds in the initial and second droplets and for ten seconds in the third droplet after a 10-minute incubation in Equilibration Solution (7.5% DMSO and 7.5% EG in a HEPES-

buffered medium). The embryos were moved to the fourth droplet, and they were then instantly placed inside a CBS-VIT-HS straw with a tiny volume. After being plunged, the straw was closed and placed in liquid nitrogen. Less than 90 seconds were needed for the whole vitrification process, from the initial droplet through the plunge into liquid nitrogen. The liquid nitrogen vapor phase was used to keep the embryos safe in a container. After freezing, the embryos were transferred to a culture dish, and their development was evaluated. At least 50% of the cells needed to be intact after thawing for survival to be considered. If all blastomeres survived, the embryo was said to be entirely whole. After overnight culture, it was deemed additional cleavage if the number of blastomeres increased. The straws were warmed until there were enough embryos for the transfer [13].

IV. Timing Of Preservation And Safety

To enhance the security and effectiveness of ovarian stimulation cycles during IVF therapy and to enable fertility preservation, cryopreservation of the oocytes as well as the embryos successfully is very crucial. In addition to protecting fertility, ovarian tissue transplantation benefits young women undergoing cancer treatment by regaining their endocrine function [23].

Slow freezing and vitrification techniques are both effective for cryopreservation. The main variations are the number of CPAs present, and the cooling speeds used for the two. Theoretically, cells might efflux internal water too quickly and enough to prevent the method of super-cooling and the formation of ice within cells if cooling is sufficiently slow. The optimal cooling speeds will differ depending on how well different cell types can transport water across their plasma membranes [24].

First, the Slow-freezing technique replaces the cytoplasmic water with CPAs, reducing cell damage and adjusting the cooling rate in line with cell membrane permeability. The utilization of a costly controlled-rate freezer or a benchtop portable freezing container, with a typical cooling rate of 1 °C/min in the presence of less than 1.0M of CPA, is required for slow-cooling methods. Since excessive dehydration will raise the intracellular concentrations of cryoprotectants to toxic levels, there must be a delicate balance between the rate of water loss from the cell and the formation of extracellular ice crystals. Slow freezing provides the benefits of minimal manipulation skill requirements and little danger of contamination during processes. Since extracellular ice forms during gradual freezing, there is a considerable danger of freezing damage. In contrast to the slow-freezing method, vitrification involves directly exposing cell suspensions to liquid nitrogen, which causes them to change from an aqueous to a glassy state. After being exposed to high concentrations of CPA (in the range of 40–60%, weight/volume), the cells or tissues must be cooled to deep cryogenic temperatures (i.e., with liquid nitrogen), followed by a quick cooling step to prevent ice nucleation. Three variables primarily affect vitrification: (1) sample viscosity; (2) chilling and warming rates; and (3) sample volume. Thus, a delicate balance must be kept between all the pertinent factors to ensure successful vitrification. Vitrification seems more appealing Because it does not require expensive equipment, only uses a small amount of liquid nitrogen, and does not take long. The need for employing highly concentrated cryoprotective solutions, which could cause osmotic shock and impair embryo viability, is the main concern ([24], [25]).

The insignificant risk of freezing damage during vitrification, which guarantees a high enough cell survival rate, is one of the process' main benefits. However, it requires good manipulation skills due to the substantial risk of contamination with pathogenic agents. The survival rate after thawing is one of the most critical measures in any oocyte cryopreservation program ([24],[27]).

No significant differences were found in embryologic and clinical outcomes, including cryo survival rate when comparing closed and open vitrification methods. The clinical pregnancy rate did not significantly differ across the groups, and similarly, in the rates of fertilization, miscarriage, continued pregnancy, or live birth [26]. The use of vitrification for embryo cryopreservation is of reassurance due to its superiority in live birth rates and non-inferiority in safety perinatal outcomes. Future studies are nevertheless required to determine the long-term effects of the vitrification method on the health of the offspring [14].

However, considering certain histological and morphological factors, the greater follicle preservation shows that the standard slow freezing method is superior to the vitrification method for the cryopreservation and transplanting of ovarian tissue, enhanced follicular cell proliferation and angiogenesis, and decreased DNA damage [23].

Table 1: Comparison between the slow-freezing and vitrification methods.

Characteristic	Procedure	
	Slow freezing	***Vitrification***
Processing Time	over three hours	Quickly, within ten minutes
Expense	pricey, require freezing machine	Cheap; no special equipment required
Sample volume (μL)	100–250	1–2
CPA concentration	Low	High
Potential for freeze damage, along with the growth of ice crystals	High	Low
Post-thaw viability	High	High
Risk of toxicity of CPA	Low	High
Status of system	Closed system only	Opened or closed system
Possibility of harmful contamination	Low	High
Dexterity required	Easy	Difficult

V. Result: Post-thawing survival rate in embryos in slow freezing Vs vitrification

Previous studies indicate that slow freezing slows down embryo metabolism as compared to vitrification. The post-thawing survival rate and the number of intact blastomeres were greater when human embryos were cryopreserved by vitrification ([13],[28]). In accordance with this, a clinical study revealed that vitrification had less detrimental effects than slow-freezing because frozen-thawed procedures conducted on human embryos and subjected to slow-freezing procedures resulted in impaired morphology, survival rates, and pregnancy rates in comparison to vitrification [18].

In a prospective randomized trial that included 3 study groups, 2 vitrification and a slow freezing group, 1055 embryos in total were warmed; 836 (79.2%) of them sustained, and 676 (64%) were eventually shifted. The survival rate of post-warming embryos was significantly higher following vitrification than following slow freezing. When we look at the outcome parameters, the proportion of preserved embryos, completely unaltered and cleaved embryos were higher in both vitrification groups than in the slow freezing group [11].

Also, there were greater transferrable embryos in the vitrification group, even though there was no substantial variance in the implantation rate. But for statistical significance the authors needed more than 2500 embryos. The article compares the effectiveness of vitrification, slow freezing, and ultra-rapid freezing in the cryopreservation of embryos using the findings of a meta-analysis of six randomized experiments. Based on the study, vitrification had a higher implantation potential, higher medical pregnancy levels, and a higher embryo lifespan. Although there was scarce information available on this technique, the study likewise indicated that ultra-rapid freezing was not as effective as gradual freezing. The post-thaw development rate and

morphological survival were two key performance indicators for cryopreservation that the Alpha consensus society identified and established benchmark KPI values for indicators. The study also discovered that for biopsy-derived embryos cryopreserved at the blastocyst stage, vitrification was superior to slow freezing. Overall, the findings point to vitrification as the most effective approach for embryo cryopreservation; nevertheless, more research is needed to fully understand the impact of these procedures on newborn outcomes and congenital anomalies ([21],[29]). In another meta-analysis which included four research the survival rate of cleavage-stage embryos and blastocyst development utilizing vitrification and slow freezing procedures. The study discovered that vitrification had higher post-thawing survival rates than slow freezing for both cleavage-stage embryos and blastocysts. Also, compared to slow freezing, vitrification accelerated blastocyst formation in fertilized eggs preserved at the cleavage stage, after thawing. The study was unable to fully compare clinical outcomes due to the minimal number of studies. According to Lee et al.'s research, vitrification is a safe way to retain blastocysts created using IVM techniques. According to a study conducted by Lee and colleagues, blastocysts can be securely preserved in a way similar to how they were generated in vitro. This procedure, which employs in vitro maturation processes, could aid in the treatment of reproductive disorders. The rates of implantation and clinical pregnancy after the surgery were comparable to those of fresh cycles, according to the researchers ([22],[30]).

VI. Summary

The process of extracting, freezing, and storing oocytes is known as “oocyte cryopreservation,” and it is essential to ART. Slow freezing procedures have been utilized to limit oocyte ice formation, although this strategy has demonstrated have significant impact on post-thaw survival rates and overall success rates of ART. High, toxic solute concentration and impaired morphology have also been observed in slow freezing. Vitrification of oocytes on the other hand, is gaining favor in IVF clinics globally being preferred for egg cryogenic preservation. Cryoprotectants are employed to harden the oocytes into an amorphous glass like substance, totally preventing ice formation and resultant hazardous solute concentrations. It has been shown that vitrification improves clinical fertilization outcomes, post-thaw survival, and cryosurvival rates in comparison to slow freezing. Moreover, vitrification technics allows for brief quarantine of donor eggs to screen for transmissible illnesses and delivers equaling compared to fresh oocytes, IVF results. Vitrification is not widely used yet because safer vitrification equipment and less cytotoxic cryoprotectants are still being developed. Inconclusion, future innovations in cryoprotectants and technologies will surely lead to the widespread implementation of vitrification procedures.

VII. Conclusion

Vitrification is faster and more desirable than slow freezing in cryopreserving embryos. This procedure provides greater clinical pregnancy rates and implantation potential along with higher survival rates, intact blastomeres, and transferrable embryos. The benchmark values for important performance measures have been determined by the Alpha consensus society, and they prefer vitrification over slow freezing. Moreover, studies have shown that vitrification, which has the likelihood of achieving a viable pregnancy and successful implantation rates equivalent to fresh cycles, is a useful technique for freezing blastocysts acquired from IVM programs. To evaluate newborn outcomes and congenital anomalies related to vitrification, however, more research is required.

VIII. Author contributions

Literature search and manuscripts formation was aided by Aishwarya Grace Unnikrishnan, Navneeth Ranjith, Afifa Khaleelullah, Madiha Bano Rehmani, Arjun Jay Prakash, Nazneen Naveed Sait. Result, summary and manuscript editing was done by Maryam Baqri and Aishwarya Grace Unnikrishnan.

References

[1]. Rivas Leonel EC, Lucci CM, Amorim CA. Cryopreservation of Human Ovarian Tissue: A Review. Transfusion Medicine and Hemotherapy. 2019;46(3):173–81.

[2]. Argyle CE, Harper JC, Davies MC. Oocyte cryopreservation: where are we now? Hum Reprod Update. 2016 Jun;22(4):440–9.

[3]. Iussig B MRFGBSVACDUFRL. 2019 M 8. A brief history of oocyte cryopreservation: arguments and facts. Acta obstetricia et gynecologica Scandinavica. 2019.

[4]. Tavukcuoglu S, Al-Azawi T, Khaki AA, Al-Hasani S. Is vitrification standard method of cryopreservation. Middle East Fertil Soc J. 2012 Sep;17(3):152–6.

[5]. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2016 Nov 8.

[6]. Gunnala V, Schattman G. Oocyte vitrification for elective fertility preservation: the past, present, and future. Curr Opin Obstet Gynecol. 2017 Feb;29(1):59–63.

[7]. Saragusty J, Arav A. Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. REPRODUCTION. 2011 Jan;141(1):1–19.

[8]. Arav A, Natan Y. The Near Future of Vitrification of Oocytes and Embryos: Looking into Past Experience and Planning into the Future. Transfusion Medicine and Hemotherapy. 2019;46(3):182–7.

[9]. Chian RC, Wang Y, Li YR. Oocyte vitrification: advances, progress, and future goals. J Assist Reprod Genet. 2014 Apr;31(4):411–20.

[10]. Li Y, Chen Z jiang, Yang H jun, Zhong W xia, Ma S ying, Li M. [Comparison of vitrification and slow-freezing of human day 3 cleavage stage embryos: post-vitrification development and pregnancy outcomes]. Zhonghua Fu Chan Ke Za Zhi. 2007 Nov;42(11):753–5.

[11]. Fasano G, Fontenelle N, Vannin AS, Biramane J, Devreker F, Englert Y, et al. A randomized controlled trial comparing two vitrification methods versus slow-freezing for cryopreservation of human cleavage stage embryos. J Assist Reprod Genet. 2014 Feb;31(2):241–7.

[12]. Sifer C, Sermondade N, Dupont C, Poncelet C, Cédrin-Durnerin I, Hugues JN, et al. Issue de la vitrification des embryons précoces versus congélation lente. Rapport de la première naissance française. Gynecol Obstet Fertil. 2012 Mar;40(3):158–61.

[13]. Balaban B, Urman B, Ata B, Isiklar A, Larman MG, Hamilton R, et al. A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation. Human Reproduction. 2008 Jun 20;23(9):1976–82.

[14]. Gu F, Li S, Zheng L, Gu J, Li T, Du H, et al. Perinatal outcomes of singletons following vitrification versus slow-freezing of embryos: a multicenter cohort study using propensity score analysis. Hum Reprod. 2019 Sep 29;34(9):1788–98.

[15]. Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. Hum Reprod. 2014 Dec;29(12):2794–801.

[16]. Van Landuyt L, Van de Velde H, De Vos A, Haentjens P, Blockeel C, Tournaye H, et al. Influence of cell loss after vitrification or slow-freezing on further in vitro development and implantation of human Day 3 embryos. Human Reproduction. 2013 Nov 1;28(11):2943–9.

[17]. Kolibianakis EM, Venetis CA, Tarlatzis BC. Cryopreservation of human embryos by vitrification or slow freezing: which one is better? Curr Opin Obstet Gynecol. 2009 Jun;21(3):270–4.

[18]. Rezazadeh Valojerdi M, Eftekhari-Yazdi P, Karimian L, Hassani F, Movaghar B. Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos. J Assist Reprod Genet. 2009 Jun;26(6):347–54.

[19]. Mastenbroek S, van der Veen F, Aflatoonian A, Shapiro B, Bossuyt P, Repping S. Embryo selection in IVF. Human Reproduction. 2011 May 1;26(5):964–6.

[20]. Debrock S, Peeraer K, Fernandez Gallardo E, De Neubourg D, Spiessens C, D'Hooghe TM. Vitrification of cleavage stage day 3 embryos results in higher live birth rates than conventional slow freezing: RCT. Human Reproduction. 2015 Aug;30(8):1820–30.

[21]. AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. Reprod Biomed Online. 2010 Feb;20(2):209–22.

[22]. Amorim CA, Curaba M, Van Langendonckt A, Dolmans MM, Donnez J. Vitrification as an alternative means of cryopreserving ovarian tissue. Reprod Biomed Online. 2011 Aug;23(2):160–86.

[23]. Lee S, Ryu KJ, Kim B, Kang D, Kim YY, Kim T. Comparison between Slow Freezing and Vitrification for Human Ovarian Tissue Cryopreservation and Xenotransplantation. Int J Mol Sci. 2019 Jul 8;20(13).

[24]. Jang TH, Park SC, Yang JH, Kim JY, Seok JH, Park US, et al. Cryopreservation and its clinical applications. Integr Med Res. 2017 Mar;6(1):12–8.

[25]. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril. 2008 Jul;90(1):186–93.

[26]. Cai H, Niringiyumukiza JD, Li Y, Lai Q, Jia Y, Su P, et al. Open versus closed vitrification system of human oocytes and embryos: a systematic review and meta-analysis of embryologic and clinical outcomes. Reprod Biol Endocrinol. 2018 Dec 6;16(1):123.

[27]. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo, and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2016 Nov 8.

[28]. Shi Q, Xie Y, Wang Y, Li S. Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis. Sci Rep. 2017 Aug 17;7(1):8538.

[29]. Medicine AS in R. The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting. Reprod Biomed Online. 2012 Aug;25(2):146–67.

[30]. Son WY, Tan SL. Comparison between slow freezing and vitrification for human embryos. Expert Rev Med Devices. 2009 Jan 9;6(1):1–7.