

Reviews

Methods to improve frozen-thawed blastocyst transfer outcomes- the IVF laboratory perspective

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During the last few years, the number of frozen-thawed embryo transfer cycles (FET) significantly increased due to the universal application of more efficient cryopreservation techniques in the IVF laboratory and the improved survival rates of blastocyst stage embryos and the wide implementation of “freeze all” IVF cycles to prevent OHSS, or for preimplantation genetic testing for aneuploidy (PGT-A). Blastocyst cryopreservation allows single embryo transfers to reduce the rate of multiple pregnancies and improve perinatal outcomes. There is no consensus regarding the optimal laboratory protocol for blastocyst cryopreservation, and research is ongoing for its amelioration. This review summarizes different laboratory methods that may improve frozen-thawed blastocyst embryo transfer outcomes, alone or in combination. Some of the techniques relate to embryo survival; some of them work on endometrial receptivity.

1. INTRODUCTION

Frozen-thawed blastocyst transfer became very popular in the last decade. It provides important advantages for women who produce large numbers of oocytes and embryos in their fresh in vitro fertilization (IVF) cycle.^{1,2} This method allows to decrease the risk of multiple pregnancies³ to avoid ovarian hyperstimulation syndrome⁴⁻⁶; is considered as cost-effective⁶ and allows to decrease the stress of couples before the IVF procedure^{1,7}; allows preimplantation genetic testing (PGT) for couples with a genetic disease⁸; to increase pregnancy rates^{9,10} and the take-home baby rate.^{11,12}

Many clinical and embryological factors may affect frozen-thawed embryo transfer outcomes. Clinical factors, including female age and the etiology of infertility, affect the outcome of frozen-thawed embryo transfer.¹³ Embryological factors include embryo quality, day of freezing, and freezing/thawing laboratory protocol.^{14,15} The main problems of this technique are possible adverse effects of the freeze-thawing process, such as ice crystal formation, on the embryo. They may diminish embryo survival and pregnancy rates.

The main goal of our review is to summarize and discuss different interventions, related to the IVF laboratory, to improve the pregnancy outcome of a cryopreserved embryo transfer cycle.

THE ARTIFICIAL COLLAPSE OF THE BLASTOCYST

The process of freezing and thawing may have an adverse effect on the blastocyst. The introduction of the vitrification method¹⁶ allows to avoid intracellular ice-crystal formation compared with slow controlled freezing. Still, little ice formation may occur due to the large fluid-filled cavity in the expanded blastocysts that inhibits sufficient permeation of cryoprotectants inside the blastocoel. This problem led to developing techniques for the mechanical emptying fluid from the blastocoel. The artificial shrinkage or artificial collapsing of the blastocyst can be achieved by various techniques, including micro-needle puncture,¹⁷ blastocoel fluid aspiration¹⁸ or a laser pulse between two trophectoderm cells.¹⁹

Several authors have reported that artificial shrinkage before the vitrification of blastocysts increased survival rates and improved the clinical success rates of cryopreserved blastocyst cycles.¹⁹⁻²³ Iwayama et al., 2011 reported a similar survival rate after thawing but an increased implantation rate of collapsed blastocysts compared with non-collapsed blastocysts.²⁴ Furthermore, thawed blastocysts tended to re-expand faster after undergoing artificial shrinkage before vitrification.^{25,26}

A trend towards better clinical outcomes has been observed in two prospective randomized clinical trials.^{27,28} According to Gala et al., 2014, The survival rate in the artificial shrinkage group was significantly higher compared

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with the control group: 99% and 91.8% respectively ($P=0.01$).²⁷

Van Landuyt et al., 2015 showed that the survival rate after the collapse was significantly higher than the control group (98.0 versus 92.0%, OR: 4.25; 95% CI: 1.19–15.21). Moreover, a higher percentage of high-quality blastocysts (36.3 versus 23.5%, OR: 1.86; 95% CI: 1.12–3.08) and hatching blastocysts (19.2 versus 5.4%, OR: 4.18; 95% CI: 1.84–9.52) were found compared with the control group.²⁸

The last systematic review and meta-analysis²⁹ included eight studies. They found that blastocyst survival (OR 5.04, 95% CI 2.43–10.46) and clinical pregnancy rate (OR 1.87, 95% CI 1.26–2.77) were significantly higher in collapsed blastocysts compared to the control group. However, both groups were comparable in the implantation rate (OR 2.50, 95% CI 0.67–9.28) and live birth rate (OR 1.35, 95% CI 0.88–2.09). In conclusion, this systematic review and meta-analysis suggest that artificial shrinkage before blastocyst vitrification improves survival and clinical pregnancy rate but not implantation or live birth rate.²⁹

Despite positive results from different groups, the Alpha consensus meeting on cryopreservation did not issue any recommendations regarding artificial blastocoel collapse.³⁰ Presently, there is a lack of evidence as to whether blastocysts benefit from artificial shrinkage before vitrification, and more prospective randomized studies are needed to improve the level of evidence.

ASSISTED HATCHING

Assisted hatching (AH) is a technique used to assist artificial blastocyst hatching from the zona pellucida (ZP). During the natural development of blastocyst, the ZP is thinned, finally creating a hole, allowing the embryo to escape from the ZP. This phenomenon is essential in implantation and is called hatching. The laboratory conditions during IVF treatments, such as in vitro culture and freeze/thaw techniques, cause ZP hardening and reduce ZP lysis, hence making it more difficult for the embryo to escape from the ZP.^{31,32} Many techniques for AH had been suggested including chemical acidified Tyrode solution,^{33,34} mechanical partial ZP dissection with a glass microneedle,³⁵ and the use of a laser system.³⁶ Due to the safety and simplicity of laser procedure, many IVF laboratories prefer to use the laser-assisted hatching (LAH) method for the ART technologies. Over the years, many articles have described the benefits of AH for couples undergoing IVF procedures,^{37–40} but only a few studies have reported the live birth rate (LBR).⁴¹

According to the meta-analysis by Li et al., 2016, AH was associated with an increased chance of achieving clinical pregnancy (OR = 1.16, 95% CI = 1.00–1.36, $I(2) = 48.3\%$) and multiple pregnancies (OR = 1.50, 95% CI = 1.11–2.01, $I(2) = 44.0\%$) compared to the control.³⁷

The meta-analysis by Zeng et al., 2018, demonstrated that LAH is related to a higher clinical pregnancy rate, embryo implantation rate, and multiple pregnancy rate in women with cryopreserved-thawed embryos. However, LAH is unlikely to increase live birth rates or decrease miscarriage rates.³⁸

The study by Elnahas et al. (2018) also reported statistically increased implantation and pregnancy rates from thawed embryos submitted to assist hatching.³⁹

Other group showed that zona pellucida (ZP) thinning via LAH, performed in FET cycles, significantly improves clinical outcomes, particularly clinical pregnancy, and implantation rates, among patients with previous repeated failures.⁴⁰

Kanyo et al.⁴¹ included 413 patients of different ages with recurrent implantation failure, who were allocated randomly into LAH and control groups. They showed a significant increase in pregnancy rates ($P = 0.03$) for patients older than 37 years in the LAH group. The authors suggested performing assisted hatching on frozen embryos as a routine strategy before FET in older woman, to overcome the negative effect of zona hardening in order to increase the possibility of pregnancy achievement.⁴¹

In a recent study, Endo et al., 2021 showed that LAH has clear benefits for clinical outcomes. Performance of LAH resulted in a significant increase in the implantation rate (46.0% vs 35.6%), clinical pregnancy rate (40.8% vs 29.4%), and live birth rate (34.3% vs 22.5%) relative to the control group without hatching.⁴²

According to The Practice Committee of the American Society for Reproductive Medicine (ASRM), AH procedures could improve the clinical pregnancy rate (CPR). They suggested that individual assisted reproductive technology programs should evaluate their own unique patient populations in order to determine which subgroups may benefit from AH.⁴³

DAY 3 THAWING AND CULTURING TO BLASTOCYST

Studies on the efficacy of day 3 cleavage stage embryos thawing and their prolonged culture for 48 hours to the blastocyst stage before ET are scarce. Some studies focused on the comparison of a short culture for 3–5 hours with overnight culture.

Joshi et al., 2010 showed in a retrospective study of 518 FET cycles, that overnight culture of cleavage stage embryos resulted in an improved outcome if the embryo resumed cleavage after thawing, compared with embryos transferred within 2 h of thawing and or ET of embryos that did not resume cleavage after an overnight culture.⁴⁴

Eftekhari et al., 2012 reported in a prospective study of 134 FET, that blastocyst formation after thawing of cleavage stage embryos was a good predictor for embryo viability and pregnancy outcome.⁴⁵

According to Fernandes et al., 2013 the culture of post-thaw cleavage stage embryos to blastocyst improves implantation rate (47.5% vs 18.4%) and clinical pregnancy rate (37.3% vs 15.5%) in patients undergoing a frozen blastocyst transfer cycle compared with frozen cleavage stage embryos transferred immediately.⁴⁶

A retrospective, observational cohort study performed at two assisted reproductive technology centers, 2014 – 2020 showed results supporting the post-thaw culture of cleavage embryos (day 3) for 2 days and transferring them as blastocysts, increasing the rates of ongoing pregnancy and delivery.⁴⁷

According to a recent study, implantation rates (28.9% vs 22.4%) and clinical pregnancy rates (37.2% vs 27.9%) were higher in the group with thawed cleavage stage embryos that were cultured for 2 days and transferred as blastocysts than in the group with thawed blastocysts transfers. Live birth and abortion rates were similar in both groups.⁴⁸ Nevertheless, in patients in whom surplus thawed cleaved embryos were grown to the blastocyst stage, re-frozen, and then re-thawed for the transfer, a low pregnancy rate was observed.⁴⁹

There is no recommendation of ASRM⁴³ to thaw and culture cleavage stage embryos to blastocyst.

EMBRYO GLUE

Hyaluronan (HA), also known as hyaluronic acid, exists in the oviduct, uterus and cervix⁵⁰ and is also produced by granulosa cells in the ovarian follicles.⁵¹ HA acts through CD-44 and RHAMM receptors activating MAP kinases and Akt signaling.^{52,53} The addition of HA to embryo culture media seems to add an advantage for both IVF and ET processes.⁵⁴⁻⁵⁶

The enrichment of transfer medium with HA, known as EmbryoGlue, increases clinical pregnancy rate (CPR) and implantation rate (IR), both for day 3 and day 5 embryo transfers. The beneficial effect was most evident in women who were >35 years of age, in women who had only poor-quality embryos available for transfer, and in women who had previous implantation failures.⁵⁷

Different studies showed variable results concerning the use of hyaluronan-rich transfer media. Schoolcraft et al., 2002 found that an increased concentration of hyaluronan in an embryo transfer medium improved the implantation rate after the transfer of fresh day 3 embryos.⁵⁸

Many articles failed to show an advantage of the use of HA on IVF procedures.⁵⁹⁻⁶² Karimian et al., 2004 and Loutradi et al., 2007 failed to demonstrate a significant increase in implantation or pregnancy rate after the use of HA, although these rates were higher in the HA group.^{59, 60} Among 120 cases, no statistical difference was found between clinical pregnancies in a control group compared to a test group using EmbryoGlue (38% vs 42%).⁶¹ According to a recent randomized controlled blinded trial, there was no statistical difference in LBR between the control or EmbryoGlue culture groups in fresh/ frozen, and cleavage/ blastocyst stage transfers.⁶²

On the other hand, many studies have described the benefits of HA for couples undergoing IVF procedures.⁶³⁻⁶⁷ Valorjedi et al., 2006 in a prospective, randomized clinical trial showed that using hyaluronan-rich transfer medium significantly improved implantation and pregnancy rates in women after a previous implantation failure.⁶³ The addition of HA to transfer media for human frozen embryos significantly increased the implantation rate without increasing the delivery rate.⁶⁴ Korosec et al., (2007) reported a significantly higher pregnancy rate with hyaluronan addition in SET of the blastocyst in a group that had ≥ 2 blastocysts development and with a previous implantation failure (55% versus 10%; $P = 0.012$). Overall pregnancy rates after

fresh elective and frozen-thawed single blastocyst transfer were similar in both study and control groups.⁶⁵

According to a Cochrane Systematic Review,⁶⁶ which analyzed seventeen studies with a total of 3898 participants, improved clinical pregnancy rate and live birth rate were found with the use of functional concentrations (e.g. 0.5 mg/ml HA) of HA as an adherence compound in ART cycles. In addition, Heymann et al., 2020 showed that adding HA to transfer media resulted in an increase in both clinical pregnancy (RR 1.16, 95% CI 1.09 to 1.23; 17 studies, $N = 5247$; $I^2 = 40\%$; moderate-quality evidence) and multiple pregnancy rates (RR 1.45, 95% CI 1.24 to 1.70; 7 studies, $N = 3337$; $I^2 = 36\%$; moderate-quality evidence).⁶⁷

According to Adeniyi et al., 2020, the use of HA-rich medium for ET was positively and significantly associated with an improved clinical pregnancy rate and LBR.⁶⁸

Presently, there is a lack of sufficient evidence as to whether frozen-thawed blastocysts benefit from an addition of HA to transfer medium, and more randomized studies are needed.

GM-CSF

Various studies have suggested that soluble proteins and their receptors play an important role in the interaction between embryo and endometrium and are very important for embryo growth and implantation.^{69,70} A lot of effort was invested for trying to determine new proteins secreted by the embryo in order to contribute for a better understanding of mechanisms and interactions.^{69,71}

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a hematopoietic cytokine with proliferation, differentiation, and adhesion roles.⁷² In the female reproductive tract, GM-CSF promotes embryo implantation and pregnancy by regulating the uterine leukocyte population.⁷³⁻⁷⁵ In addition, preimplantation embryos could secrete GM-CSF.⁷⁰ In the IVF laboratory, GM-CSF concentration in the culture medium may affect pregnancy outcomes.⁷⁶ In comparison of implanted and non-implanted blastocyst conditioned medium, a difference was observed in GM-CSF with higher level in implanted blastocysts. The discussion from this study suggested that the implanting blastocyst communicates with maternal cells by binding or metabolizing the essential proteins secreted by the endometrium, such as GM-CSF, to begin the implantation process.⁷⁶

Recent several studies suggest that the addition of GM-CSF into the culture medium improves human and mouse embryo quality.^{77,78} In addition, GM-CSF receptors are expressed on the surface of human and mouse embryos,⁷³ and this fact may explain the finding that GM-CSF can enhance cell survival and prevent apoptosis in frozen-thawed embryos.⁷⁹

Chen et al., 2021 reported the presence of GM-CSF in a single-blastocyst conditioned medium (SBCM) and showed that GM-CSF concentration was correlated with embryo quality and pregnancy rates. The author's conclusion was that GM-CSF detection may be used as a biomarker for the prediction of pregnancy outcomes. This may help in the selection of embryos with a high potential to implant.⁷¹

According to the study by Okabe-Kinoshita (2022) the use of a GM-CSF-containing medium for blastocyst-recovery culture improved the live birth rate because of increased implantation rate in the frozen-thawed blastocyst-transfer cycle. In patients who underwent frozen-thawed blastocyst transfer with GM-CSF, the percentage of β HCG positive cases, biochemical pregnancies, clinical pregnancies, ongoing pregnancies, and live birth rates was 60.6%, 7.98%, 52.6%, 42.9%, and 40.9%, respectively, as compared with 45.1%, 3.29%, 41.8%, 31.1%, and 30.5%, respectively, for the control groups. According to the author, the use of GM-CSF-containing medium following blastocyst thawing may be used for improving the blastocyst-transfer outcomes.⁸⁰

Presently, there are only a few studies advocating the benefits of GMCSF for IVF success due to the recent discovery of this protein, therefore, more randomized studies are needed.

MORPHOLOGY AND SURVIVAL GRADING

Vitrification has been applied to embryos at both the cleavage and blastocyst stages, and clinical studies have shown high survival and promising implantation rates after the transfer of thawed embryos at all.⁸¹⁻⁸³

Data on the safety of vitrification for obstetric and perinatal outcomes are also reassuring.^{81,84} However, blastocysts are unique because their multicellular structure and the presence of water-filled blastocoele make it difficult to achieve the required level of dehydration and high viscosity uniformly across all blastomeres.⁸⁵ The blastocyst undergoes cooling under the influence of cryoprotectants, followed by warming under the influence of cryoprotectant removal. Cell damage or even cell death could be the outcome of the blastocoele's swelling and shrinking. When compared to a fresh blastocyst, it becomes challenging to assess blastocyst quality.

Animal models have shown that inadequate intracellular delivery of cryoprotectants and inadequate dehydration of blastocysts can lead to decreased survival and increased apoptosis.⁸⁶ Different studies reported ultrastructural damage to mitochondria and cytoskeleton in vitrified blastocysts, and altered methylation after vitrification.⁸⁶⁻⁸⁸ Most studies on human vitrified blastocysts have focused on evaluating the success of the procedure based on post-thaw survival and post-implantation clinical outcomes. Only a few examined dividing cells and the possible effects of vitrification on spindle structure, chromosome alignment, and the ability of spindles to form and continue normal cell division.⁸⁶

According to Chatzimeletiou et al., 2011, an examination using confocal laser scanning microscopy revealed a significantly higher incidence of mitotic spindle abnormalities in the vitrified group compared to the fresh group which may lead to chromosomal mosaicism.⁸⁹ The biomarkers of the viability and integrity of the thawed embryo are the embryo morphology appearance and viability.

The combination of morphology and viability scores has been shown to be more precise in predicting implantation outcome than has the morphology score alone^{90,91}; However, reported data on the prediction of implantation out-

come in FET cycles are relatively rare.^{92,93} According to, Li et al., 2015, the mean viability index of the 0 % implantation group was significantly lower than that of the 100 % implantation group. This was consistent with previous results, which indicated that the relative viability scores were statistically significantly different between positive and negative pregnancy outcomes after frozen-thawed embryo transfer.⁹²⁻⁹⁴ Another study shows that the live birth rate (LBR) increased from 6.5% for blastocysts with a very low cell survival rate to 34.7% for blastocysts with a high cell survival rate ($p = 0.001$). LBR was 6.7% in blastocysts with the worst morphological characteristics after warming- blastocysts that collapsed and had very low cell viability. Post-warming high cell survival and good morphology are associated with higher LBR in euploid and untested blastocysts.⁹⁵

Further studies are needed to elucidate the mitotic stages that are susceptible to damage during vitrification and the potential impact these may have on the chromosome organization of developing blastocysts.

BLASTOCOEL RE-EXPANSION

The blastocoele expansion was found to correlate with cycle outcome in vitrified-warmed embryo transfer cycles.^{95,96}

The method to assess blastocoele re-expansion, which may occur within 1 to 2 hours after the warming, is provided by the protracted culture of post-warming blastocysts.^{97,98} The literature published concerning the relationships between blastocoele re-expansion speed and pregnancy outcomes of vitrified-warmed blastocyst transfers is scarce.

Shu et al.⁹⁹ showed that embryo transfer cycles with at least one rapidly re-expanding blastocyst had almost two times the CPR and IR of those with no rapidly re-expanding blastocyst. This shows that growing early blastocysts to the enlarged blastocyst stage before freezing them can increase their tolerance to cryopreservation chemicals and make it easier for the blastocysts to survive and re-expand again after warming. In comparison to enlarged blastocysts, those that did not significantly re-expand after warming were more vulnerable to vitrification freezing and warming and had a lower survival rate.⁹⁸ Unexpanded blastocysts 2 hours after warming may still have some chance of implanting on the endometrium, as evidenced by the fact that one ongoing clinical pregnancy was established from 10 transferred blastocysts that did not exhibit blastocoele re-expansion. These findings are consistent with earlier research findings, which showed that certain shrunken post-warm blastocysts may develop to the hatched stage after thawing for 5 to 6 hours and that the shrunken blastocyst exhibited sufficient implantation capacity.¹⁰⁰ Allen et al., 2022, reviewed 612 cycles, of which 196 included PGT-A embryos. They found that the live birth rate (LBR) increased from 11.4% in the collapsed blastocysts group to 38.9% in the post-warming full re-expansion group ($p < 0.001$). They summarized that post-warming re-expansion is associated with higher LBR in euploid and untested blastocysts.⁹⁵ Other studies confirm that re-expansion is associated with elevated live birth.^{101,102} Coello et al. reported

the outcome of 429 frozen-warmed blastocysts transfer cycles in patients who received egg donation. Re-expansion had a strong correlation with implantation rate.¹⁰¹

The ESHRE Embryology report on ART laboratory performance indicators selected the degree of re-expansion as the best post-thaw parameter for the prediction of live birth.

ARTIFICIAL INTELLIGENCE

During the last few years, artificial intelligence (AI) and machine learning have been used as innovative tools of research with clinical use in the field of vitro fertilization (IVF), especially focusing on the ranking and selection of embryos for transfer and cryopreservation.¹⁰³

Current AI techniques for embryo assessment have the potential to automate and ameliorate the performance of manual and subjective selection.¹⁰³⁻¹⁰⁵ This could improve clinical workflow, reduce the time spent on manual assessments, and by allowing the transfer of the most viable embryo, potentially shorten the time to achieve pregnancy. In recent years, several studies have reported promising results using artificial intelligence (AI) to automatically analyze embryo images and videos.^{104,105} The purpose of these methods is to rank embryos according to their implantation potential and predict the probability to achieve pregnancy. According to recent publications, AI classifiers have the potential of predicting live births that humans cannot predict. Artificial intelligence may make progress in assisted reproductive technology and improve its accuracy even further.¹⁰⁴⁻¹⁰⁷

There are some ethical considerations in the AI application.^{108,109} A primary principle and priority for AI programming in medicine must always be safety. In addition, the programs must be transparent, credible, auditable and recoverable.¹¹⁰ In 2022, the World Health Organization published a global report on Artificial Intelligence in health, which detailed six guiding principles for its use and design.¹¹¹ The guidelines are based on the human health-care system controlling medical decisions in addition to maintaining privacy and confidentiality. Valid informed consent must be provided by patients through appropriate legal data protection frameworks.¹¹¹

In order to ensure that human-centered AI improves and enhances rather than replaces human skills and capabilities, all stakeholders must make a concerted effort.

AI models were presented at ASRM and ESHRE 2018 with emphasis on their ability to identify embryos with the highest fertility potential and newer algorithms are developed to achieve better results. Surely, before their incorporation into the routine laboratory workflow, AI models need to be trained and validated.

AI is clearly an emerging technique in human reproduction and embryology with high potential benefits to IVF laboratory efficacy.

3. DISCUSSION AND CONCLUSIONS

Vitrification and warming processes still present major challenges for embryos. Dehydration and rehydration can cause cell damage. In addition, there is a potential risk of structural damage due to ice crystal formation due to the presence of large amounts of liquid within the embryonic cavity. It has been noticed that after vitrification, ultra-structural damage to mitochondria and cytoskeleton, and altered methylation in vitrified blastocysts, might appear.

In this review, different laboratory interventions to improve the pregnancy outcome of a cryopreserved embryo transfer cycle were summarized.

Supporting evidence was presented indicating that artificial collapse may benefit embryos survival rate.

Data was presented showing that assisted hatching may be beneficial to overcome zona pellucida hardening after cryopreservation.

Thawing embryos on day 2/3 and culturing to blastocyst may be used to increase the pregnancy rate.

Different ways to improve endometrial receptivity by the addition of Hyaluronic acid or GMCSF to culture or embryo transfer medium were presented. A summary of these data is presented in [Table 1](#).

The assessment of the viability, morphological appearance, and the re-expansion of the blastocoel play an important role in the evaluation of the thawed blastocyst and its chance to result in a viable pregnancy. It has been noticed that there is a strong correlation between fast re-expansion of the blastocoel, good viability and good morphology after the thawing process and the pregnancy rate. In addition, AI and machine learning models have the potential ability to identify embryos with the highest fertility potential. By using AI, embryologists might rank and select the best embryos for the transfer. A summary of these data is presented in [Table 1](#).

Presently, due to a lack of sufficient data, no universal rules may be suggested, and every IVF unit may choose their own technique or combine several techniques to achieve the best results.

AUTHOR CONTRIBUTIONS PER CREDIT

Conceptualization: Yulia Michailov; Methodology: Yulia Michailov; Writing - original draft preparation: Yulia Michailov, Shevach Friedler; Writing—review and editing: Bozhena Saar-Ryss; Supervision: Shevach Friedler, Bozhena Saar-Ryss, Yulia Michailov. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

None.

Table 1. Summary of different laboratory methods that affect embryo survival and IVF outcomes.

Live birth rate	Ongoing pregnancy rate	Implantation rates	Clinical pregnancy rate	Survival rate	
- Unreported	- Unreported	+ Iwayama et al.,2011 (P < 0.017)	+ Boyard et al.,2022 (OR 5.04, 95% CI 2.43-10.46)	+ Van Landuyt et al., 2015 (OR: 4.25; 95% CI: 1.19-15.21) Gala et al.,2014 (P=0.01) Boyard et al.,2022 (OR 5.04, 95% CI 2.43-10.46)	Artificial collapse
+ Endo et al., 2021 (P < .01)	- Unreported	+ Zeng et al.,2018 (OR = 1.59, 95% CI = 1.06-2.38, I ² = 82%) Elnahas et al. ,2018 Lu et al., 2019 Endo et al., 2021	+ Li et al., 2016 (OR = 1.16, 95% CI = 1.00-1.36, I ² = 48.3%) Zeng et al.,2018 (OR = 1.65, 95% CI = 1.24-2.19, I ² = 49) Elnahas et al. ,2018 Lu et al., 2019 Kanyo et al., 2016 Endo et al., 2021 (P < .01) ASRM, 2014	- Unreported	Assisted hatching
+ Rahav-Koren et al.,2021	+ Eftekhar et al., 2012 Rahav-Koren et al.,2021	+ Joshi et al., 2010 Eftekhar et al., 2012 Fernandes et al., 2013 Aytac et al.,2022	+ Joshi et al., 2010 Fernandes et al., 2013 Aytac et al.,2022	- Unreported	Day 3 thawing and Culturing
Bontekoe et al.,2014 Adeniyi et al., 2020	- Unreported	+ Urman et al., 2008 Nakagawa et al., 2012 Schoolcraft et al., 2002 Valorjedi et al., 2006 Hambiliki et al.,2010	+ Urman et al., 2008 akagawa et al., 2012 Valorjedi et al., 2006 Korosec et al., 2007 Bontekoe et al., 2014 Heymann et al.,2020 Adeniyi et al., 2020	- Unreported	Embryo glue
+ Okabe-Kinoshita et al.,2022	+ Okabe-Kinoshita et al.,2022	+ Okabe-Kinoshita et al.,2022	+ Chen et al., 2021	- Unreported	GM-CSF

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Table 2. Summary of different laboratory evaluation methods to increase FET outcomes.

Live birth rate	Ongoing pregnancy rate	Implantation rates	Clinical pregnancy rate	
- Allen et al., 2022 Coello et al 2015 Desai et al.,2016	- Unreported	+ Shu et al.,2009 Allen et al., 2022 Coello et al.,2015	+ Shu et al.,2009 Allen et al., 2022	Blastocoel re-expansion
+ Allen et al., 2022	- Unreported	+ Maezawa et al.,2014 Li et al., 2015 Hardarson et al., 2008 Fu et al.,2013	+ Li et al., 2015 Kosasa et al., 2005	Morphology and survival grading
+ Miyagi et al.,2020	- Unreported	- Unreported	- Unreported	Embryo selection by AI



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