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The Success Rate of Cryopreservation and Thawing of Embryos at Day 3 and Day 5 Following Intracytoplasmic Sperm Injection (ICSI)

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Abstract: Cryopreservation, the technique of freezing and thawing embryos, is essential in in vitro fertilization (IVF) treatments to ensure the long-term preservation of embryos for future use. This innovative study evaluates the effectiveness of these procedures conducted at a private hospital in Tangerang, Indonesia, over a period of seven years (2016-2023), involving 18,000 couples. The design research was conducted retrospectively. The study examined embryos that underwent cryopreservation and subsequent thawing, with data collected on the rates of cell division observed on Day 3 and the rates of blastocyst formation on Day 5 after thawing. The study investigated the rates of cellular division on Day 3 and the development of blastocysts on Day 5 following the thawing process. The findings demonstrated a remarkable cleavage rate of 95.24% and a blastocyst rate of 97.22%. The remarkable results underscore the efficacy of the advanced freezing and thawing techniques employed. This discovery is highly significant since there is an urgent requirement to improve the success rates of in vitro fertilization (IVF) for couples facing infertility. It offers a fresh sense of hope and increased opportunities for establishing successful pregnancies with the improvement of cryopreservation techniques.

Keywords: Cryopreservation; Embryo; Morphology; Reproductive health; Thawing

Introduction

State Embryo cryopreservation is a vital advancement in the field of assisted reproductive technology, particularly within in vitro fertilization (IVF) protocols (Blakemore et al., 2021; Claes & Stout, 2022). This technique allows for the long-term storage of embryos, offering couples struggling with infertility multiple opportunities to achieve pregnancy from a single egg retrieval cycle. This is particularly beneficial because the retrieval process can be physically demanding, emotionally taxing, and financially burdensome. By preserving embryos, couples can attempt future transfers without undergoing repeated cycles of ovarian stimulation and retrieval. This significantly improves the overall efficiency of IVF treatment and provides hope for those facing fertility challenges.

The process of embryo cryopreservation involves freezing embryos at a specific developmental stage to halt cellular activity while maintaining their potential for future growth. The key to the success of this process lies in the precision of the freezing and thawing techniques used. If not done accurately, the delicate

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structure of the embryos could be compromised, leading to a reduced chance of successful implantation and pregnancy (Capodanno et al., 2016). In fact, the success of embryo cryopreservation is heavily reliant on maintaining the viability of embryos through precise temperature control, the use of cryoprotectants to prevent ice crystal formation, and careful handling to avoid physical damage (Lai et al., 2024; Shi et al., 2024).

One of the notable advantages of embryo cryopreservation is the ability to extend the culture of embryos after thawing. Typically, embryos are frozen at the cleavage stage, which occurs on Day 3 of development. However, after thawing, these embryos can be cultured for an additional two days to reach the blastocyst stage, which occurs on Day 5. This extended culture period is significant because blastocyst-stage embryos have a higher likelihood of successful implantation when transferred to the uterus compared to cleavage-stage embryos (Elnahas et al., 2017). Research suggests that embryos which reach the blastocyst stage exhibit greater developmental potential and are more likely to result in successful pregnancies and live births (Chen et al., 2024; Ha Vuong et al., 2024).

This extended culture period allows embryologists to assess the quality of the embryos more effectively. Since not all embryos will progress to the blastocyst stage, those that do are often considered to have better viability (Garratt et al., 2024; M. Y. Wu et al., 2018). By selecting embryos that have successfully developed to the blastocyst stage, the chances of a successful pregnancy and live birth increase. This selection process improves the overall efficiency of IVF by reducing the likelihood of multiple failed transfers and offering a higher probability of success with each transfer. The enhanced ability to select viable embryos contributes to a more tailored and effective IVF process, which is critical for couples who have already invested significant time and resources into achieving pregnancy (Sciorio et al., 2024).

Moreover, the decision to transfer blastocysts instead of cleavage-stage embryos has been linked to improved pregnancy outcomes. Studies have shown that blastocyst transfers lead to higher implantation rates, increased clinical pregnancy rates, and better chances of live birth compared to cleavage-stage transfers (Sciorio & Esteves, 2020; Sun et al., 2022). This is largely due to the fact that blastocyst-stage embryos are more synchronized with the uterine environment during implantation (van Duijn et al., 2021). At this stage, the embryo is more developed and has a higher likelihood of establishing a successful connection with the endometrial lining, which is crucial for the continuation of pregnancy. However, it is important to note that not all embryos will survive the freezing and thawing process. The effectiveness of embryo cryopreservation depends on several factors, including the quality of the embryos prior to freezing, the specific cryopreservation protocol used, and the proficiency of the embryology team in handling the embryos during the process. In many cases, embryos of lower quality may not survive thawing or may fail to develop further after thawing (Sik et al., 2020). As a result, the initial quality of the embryos plays a pivotal role in determining the success of cryopreservation and subsequent IVF outcomes.

In light of these factors, the objective of this study is to evaluate the efficacy of embryo cryopreservation and thawing procedures at a private hospital in Tangerang, Indonesia, over a seven-year period (2016-2023). This long-term study aims to assess how well the cryopreservation protocols used at the hospital have performed in preserving embryo viability and contributing to successful IVF outcomes. Specifically, the study focuses on analyzing the rates of embryo cleavage on Day 3 and the development of blastocysts on Day 5 after thawing, which are key indicators of embryo viability and developmental potential (Du et al., 2017).

The data collected from this study will provide valuable insights into the effectiveness of embryo cryopreservation in this particular setting and offer a deeper understanding of the factors that influence IVF success. By examining the rates of cleavage and blastocyst development, researchers can better understand the strengths and limitations of the cryopreservation techniques used and identify potential areas for improvement(Polyzos et al., 2018). Additionally, this study could help refine embryo selection criteria, allowing for more accurate predictions of which embryos are most likely to result in successful pregnancies.

Furthermore, the findings of this study have the potential to contribute to the broader field of reproductive medicine by offering evidence-based recommendations for optimizing embryo cryopreservation practices. As cryopreservation becomes an increasingly important component of IVF treatment, understanding its impact on embryo viability and pregnancy outcomes will be essential for improving fertility care (Chang et al., 2017). The results of this study may also help inform decisions about the ideal timing of embryo transfers, as well as the use of advanced technologies such as preimplantation genetic testing, which could further enhance the success of IVF treatments.

Embryo cryopreservation plays a critical role in contemporary IVF protocols, offering couples multiple chances to achieve pregnancy from a single egg retrieval

cycle. The process of freezing and thawing embryos, while highly effective, requires precision to maintain embryo viability. Extending the culture period after thawing to allow embryos to reach the blastocyst stage can improve IVF success rates by enabling more effective embryo selection and enhancing pregnancy outcomes (Braga et al., 2016). This study will provide valuable insights into the efficacy of cryopreservation techniques at a private hospital in Tangerang, Indonesia, and contribute to the ongoing efforts to optimize IVF treatment for couples facing infertility.

Method

Study Design

This study conducted a retrospective analysis of data from 18,000 couples who received in vitro fertilization (IVF) treatments at a private hospital in Tangerang, Indonesia, from 2016 to 2023. The study examined embryos that underwent cryopreservation and subsequent thawing, with data collected on the rates of cell division observed on Day 3 and the rates of blastocyst formation on Day 5 after thawing.

Morphological Evaluation of the Embryo

The embryos were evaluated according to globally recognized morphological criteria. The cleavage rates were determined based on the proportion of embryos that successfully underwent cell division to generate numerous cells by Day 3. The assessment criteria comprised the quantification of cells, the regularity of blastomeres, and the existence or nonexistence of fragmentation. Blastocyst rates were assessed based on the proportion of embryos that successfully progressed into blastocysts by Day 5. The blastocysts were evaluated based on criteria such as the blastocoel growth, the quality of the inner cell mass (ICM), and the trophectoderm (TE) cells.

Embryo Freezing and Thawing Protocols

The Cryotop method, a very effective approach, was used to achieve embryo vitrification. This process prevents the production of ice crystals, ensuring the preservation of embryo integrity. The thawing technique entailed the swift increase in temperature and rehydration of the embryos by exposing them to a sequence of sucrose solutions with decreasing concentration.

Morphological Evaluation of Embryos

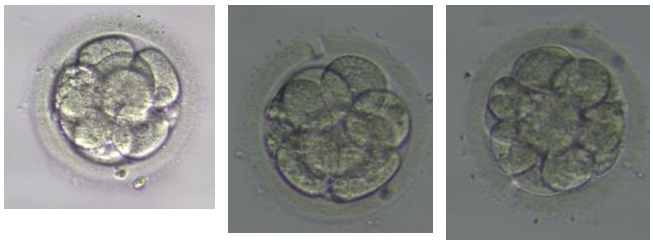
The embryos were evaluated according to globally recognized morphological criteria. Embryo morphology has been the predominant technique used by embryologists to monitor embryo development and choose the most ideal embryo(s) for transfer since the introduction of IVF. The conventional morphological examination involves assessing many criteria such as cell count, division rate, fragmentation level, multinucleation presence, blastomere size and symmetry, and zona pellucida thickness. During the blastocyst stage, the evaluation of blastocoel expansion, as well as.

Result and Discussion

Results

Cleavage rates

The research showed that 95.24% of the samples underwent cleavage on the third day after freezing. The significant rate demonstrates that the overwhelming majority of embryos successfully endured the freezing procedure and were able to progress in their growth (Figure1).

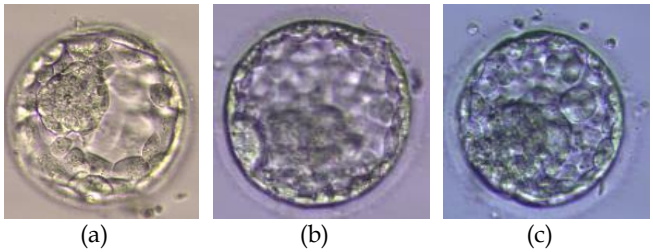


(a) Embryo before Freezing (b) Post Thawing (c) Post Culture

Figure 1. Cleavage Stage

Blastocyst rates

On the fifth day after thawing, the rate of blastocysts was determined to be 97.22%. These findings indicate that almost all embryos that reached the cleavage stage successfully progressed into blastocysts, exhibiting a high level of developmental capability. See figure 2.



(a) (b) (c)

Figure 2. Blastocyst Stage: a) Embryo before Freezing; b) Post Thawing; c) Post Culture

Discussion

Assessing the quality of embryos at the cleavage stage is a widely established method used by many embryologists for evaluating embryo quality. In order to achieve this objective, several morphological

characteristics have been proposed. The most prominent characteristics among these are: fragmentation rate (Fr), abnormalities in blastomeres, multinucleation, and the amount of blastomeres (Chen et al., 2024). The scoring of cleavage-stage embryos according to the Istanbul consensus. The consensus was reached to assign embryos a score based on four categories: A for top quality, B for good quality (not suitable for elective single-embryo transfer), C for impaired embryo quality, and D for embryos not advised for transfer (including all multinucleated embryos). Considering the substantial influence of the culture medium and culture system on embryo shape, it is imperative to consider these factors when making such comparisons. Consequently, each laboratory was prompted to create their own explanations for embryos in each of these classifications, using on current observations (Casciani et al., 2023).

Furthermore, it was evident that once an embryo had commenced its expansion phase (specifically, for blastocysts rated as 3–6, which are considered fully developed blastocysts), it became feasible to assign separate scores to the inner cell mass (ICM) and the trophectoderm (TE). The subsequent stage of the grading process was intended to be carried out using an inverted microscope(Fauque et al., 2024; Konc et al., 2014). The utilization of the grades A, B, and C was an endeavor to enhance the system's user-friendliness initially. Regarding ICM, grade A represents a densely populated ICM with numerous cells, grade B indicates a sparsely arranged ICM with numerous cells, and grade C signifies an ICM with relatively few cells. Regarding TE, grade A represents a TE consisting of numerous cells that create a tightly connected epithelium. Grade B indicates a TE with a small number of cells generating a less tightly connected epithelium. Grade C signifies a TE with a very limited number of cells. It was expected that the scoring system would be adjusted and improved once the importance of the scores was comprehended. As an illustration, a subsequent study introduced an additional letter D to indicate the existence of degenerative tissue, while another investigation incorporated ICM grades of D and E (Rodriguez-Wallberg et al., 2019).

During early human embryogenesis, a fertilized zygote proceeds through continuous cell division, cellular differentiation, and spatial organization to become a hollow spherical structure known as the blastocyst. The blastocyst is composed of three main lineages: the epiblast, trophectoderm, and hypoblast. These lineages will differentiate into the embryo itself, the placenta, and the yolk sac, respectively (El-Toukhy et al., 2017; Yurchuk et al., 2021).

Embryo cryopreservation is an essential procedure in Assisted Reproductive Technology (ART) that provides numerous advantages and caters to diverse therapeutic requirements. Embryo cryopreservation is used in two main scenarios: elective and non-elective. These scenarios have different aims and are used for different groups of patients (Nagy et al., 2020). The elective indications include oocyte donation and oocyte banking. Cryopreservation in oocyte donation eliminates the requirement for coordinating the menstrual cycles of the donor and recipient, hence streamlining the logistical aspects of the process. Additionally, it aids in addressing the significant need for donor oocytes, hence decreasing waiting lists and enhancing the availability of assisted reproductive technology (ART) services (Galli et al., 2016; Hsiao et al., 2023). Women who desire to postpone childbearing for business or personal motives have the option to retain their fertility by cryopreserving their oocytes, a process known as social freezing. This technique is especially relevant in preparation for the loss in fertility that occurs with aging. Social freezing offers a means of preserving fertility without the immediate requirement for fertilization, which might be particularly important in civilizations with specific religious, moral, or ethical concerns.

Clinical oocyte freezing, also known as oocyte cryopreservation, is a method used to collect and store oocytes (eggs) for future in IVF cycles. This technique is particularly advantageous for women who do not respond well to ovarian stimulation or experience repeated failures in implantation. Additionally, it enhances Preimplantation Genetic Testing (PGT) by offering a greater abundance of oocytes. Another indication is found in the context of transgender individuals undergoing female to male transition. Oocyte cryopreservation offers the opportunity for transgender males to safeguard their fertility prior to undergoing gender-affirming procedures. The stored oocytes can be utilized for future fertilization by a spouse or sperm donor, thereby preserving reproductive choices after undergoing transition(Lin et al., 2022).

Cryopreservation is recommended for non-elective indications, namely in cases of medical oocyte freezing. Gonadotoxic Treatments: Women undergoing fertility-compromising treatments, such as chemotherapy for cancer, can derive advantages from oocyte cryopreservation. This procedure protects their ability to reproduce from the harmful consequences of such treatments (Canel et al., 2017; C. H. Wu et al., 2015). Medical Pathologies: Medical conditions such as severe endometriosis or genetic abnormalities like Turner's syndrome, which affect fertility, require the preservation of eggs for future reproductive choices. Incidental Oocyte Freezing, also known as Emergency Freezing, is a procedure performed when sperm is not accessible on

the day of oocyte retrieval in an IVF cycle. This procedure is important to preserve the collected oocytes. Cryopreservation of surplus oocytes is possible during IVF cycles for future utilization. This procedure guarantees the availability of extra oocytes for future cycles, eliminating the need for another round of ovarian stimulation. Clinical necessities, such as oocyte accumulation, are important for patients with poor ovarian response or recurrent implantation failure. By collecting oocytes over numerous cycles, the chances of success in future in vitro fertilization (IVF) attempts are increased. This technique also facilitates the accessibility of oocytes for preimplantation genetic testing (PGT), hence increasing the likelihood of a successful pregnancy (Lin et al., 2022).

The reasons for embryo cryopreservation are based on its adaptability and crucial role in assisted reproductive technology (ART). Cryopreservation expands the possibilities for healthy pregnancies to a broader group of patients by offering solutions for both elective and non-elective requirements. This addresses both medical obstacles and individual desires in preserving fertility (Squires & McCue, 2016; Xiong et al., 2020). The study's findings are quite promising, suggesting that the embryo freezing and thawing processes used at the private hospital in Tangerang are remarkably efficient. The observed high rates of cleavage and blastocyst formation indicate that the embryos retain their capacity to survive and have the potential to successfully implant and develop during the freeze-thaw process (Schiewe et al., 2015; Ueno et al., 2014).

The employment of vitrification, a quick cryopreservation technique, is probably a substantial contributor to these outcomes. Vitrification is a process that inhibits the development of ice crystals, which have the potential to harm cellular structures. This process helps to maintain the integrity of the embryos. The success rates recorded are also influenced by the rigorous adherence to defined processes and the utilization of high-quality frozen medium (Lazzari et al., 2020; Yoshida et al., 2022). These findings are especially significant for couples undergoing IVF, as they showcase the dependability and efficacy of contemporary cryopreservation techniques. Cryopreserving embryos without sacrificing their viability offers increased adaptability and enhanced prospects for achieving successful pregnancies.

Conclusion

This study provides evidence of the great efficacy of embryo freezing and thawing methods at a private hospital in Tangerang, Indonesia. The results show a 95.24% cleavage rate on Day 3 and a 97.22% blastocyst rate on Day 5. The significant outcomes highlight the crucial role of sophisticated cryopreservation techniques and strict guidelines in improving IVF performance. The study presents a promising prospect for couples in search of reproductive treatments, emphasizing the possibility of improved success rates through enhanced cryopreservation techniques.

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Author Contributions

RM and MJ **r**esponsible for the methodology and formal analysis; RM were responsible for the investigation; MJ were responsible for the data curation; RM and MJ were responsible for the final draft. The manuscript has been read and approved by all of the writers.

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Conflicts of Interest

The authors declare no conflict of interest.

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