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Cleavage (Day 3) Vs Blastocyst (D5 and D6) Frozen Embryo Transfer

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ABSTRACT

Objectives: Knowledge about the success of the IVF program in frozen embryo transfer using cleavage stage (day 3) versus blastocyst stage (day 5 and day 6) is still limited. Several studies have found that embryo transfer at the blastocyst stage is more effective than at the cleavage stage. This study aimed to determine the quality of embryos after freezing-thawing and success rates on frozen embryo transfer on day 3 compared to day 5 and day 6.

Materials and Methods: This retrospective case control analytic study included 157 participants aged below 40 years old who met our inclusion criteria between March 2018 to December 2022. Twenty-nine patients had day 3 frozen embryo transfers with a total of 65 frozen-thawed embryos, 110 patients had day 5 frozen embryo transfers with a total of 203 frozen-thawed blastocysts, and 18 patients had day 6 frozen embryo transfers with a total of 30 frozen-thawed blastocysts. Parameters were observed based on survival rate of embryos after freezing-thawing, number of embryo transfer per groups, pregnancy, implantation, miscarriages, intrauterine fetal death (IUFD), anembryonic pregnancy, and live birth rates.

Results: There was no significant difference in survival rate of embryos after freezing-thawing among groups, however, pregnancy and implantation rates were significantly higher for day 5 frozen embryo transfer compared to those on day 3 and day 6 frozen embryo transfers. No significant differences were found in the miscarriage rate, IUFD, and live birth rate in the three groups.

Keywords: day 3 frozen embryo transfer, day 5 and day 6 frozen embryo transfer, recovery embryos, pregnancy rate, implantation rate, live birth rate

INTRODUCTION

In general, a married couple is considered infertile if they do not get pregnant within 12 months or more after having regular sexual intercourse without using contraception. Infertility is a reproductive system disorder that is not only a health problem but can also contribute to interpersonal problems. Based on the data obtained, as many as 50-80 million couples and 186 million individuals experience infertility throughout the world.[1] Meanwhile, infertility in Indonesia is estimated at around 12-22% of couples.[2] Several factors that can cause infertility are blockages in the fallopian tubes, Polycystic Ovary Syndrome (PCOS), endometriosis, and poor sperm quality and quantity.[3] Assisted Reproductive Technology (ART), which is commonly known as the IVF program, is the latest technology used to unite egg and sperm cells outside the body (*in vitro*).[4] This technology can stimulate a large number of egg cells in women, enabling an adequate number to become fertilized at the same time.

Fresh embryo transfer refers to the insertion of an embryo into the mother's uterus in the same cycle that the embryos are produced, while frozen transfer involves freezing the embryo first and then thawing it prior to insertion. After the thawing process, the embryo is transferred during a different cycle to which it was produced.[5] A frozen transfer is carried out if there are excess embryos that are not transferred at the same time, in women who have indication of Ovarian Hyperstimulation Syndrome (OHSS), clinical endometrial anomalies such as thin endometrium, polyps, elevated progesterone levels on the last day of stimulation, or having supernumerary embryos.

Frozen embryo storage is usually performed at cleavage stage (on day 3) or blastocyst stage (on day 5 or day 6).[6] Blastocysts are late-stage embryos that can be cultured *in vitro*, and can effectively increase the rate implantation and pregnancy following transfer compared to day 3 embryos. This is because, naturally, the embryo implants in the uterus at the blastocyst stage.[7] However, some studies have also reported no difference in the success rate of IVF between cleavage stage embryo transfer and blastocyst stage embryo transfers.[8,9,10] Therefore, this study aimed to determine the success rate of frozen cleavage stage transfer on day 3 compared to frozen blastocyst stage transfer on day 5 and day 6.

MATERIALS and METHODS

We conducted this retrospective case-control study at the Royal IVF Clinic, Bali Royal Hospital, Indonesia. Data was collected using medical records of all IVF patients who matched our criteria from March 2018 until December 2022. Data from 157 samples under the age of 40 years old and who undergone frozen embryo transfer were collected. The inclusion criteria in this study include data on individuals carrying out frozen embryo transfers on day 3 and day 5 and 6. The exclusion criteria in this study were incomplete medical record data, maternal age at 40 or over, or having undergone mixed blastocyst transfer on day 5 and day 6. We choose to freeze embryos on day 3 if the patients have a lower number of embryos (less than 5 good embryos on day 3), and for those with more than 5 good embryos on day 3 culture would be extended until day 5 and day 6 before freezing.

This retrospective case control analytic study included 157 participants aged below 40 years old who met our inclusion criteria between March 2018 to December 2022. Twenty-nine patients had day 3 frozen embryo transfers with a total of 65 frozen-thawed embryos, 110 patients had day 5 frozen embryo transfers with a total of 203 frozen-thawed blastocysts, and 18 patients had day 6 frozen embryo transfers with a total of 30 frozen-thawed blastocysts.

In all patients, controlled ovarian hyperstimulation was performed using short antagonist protocols. Briefly, patients were administrated recombinant FSH (rFSH, Gonal F, Serono) based on age and follicle size. Ovulation stimulation was triggered using human chorionic gonadotropin (hCG, Pregnyl; Organon, Brussels, Belgium). At 2 hours after oocyte retrieval, oocytes were denuded and all matured oocytes were inseminated by intracytoplasmic sperm injection (ICSIS). Fertilized oocytes were cultured for 3 days in G1 culture medium (G1, VitroLife, Gothenburg, Sweden), and extended culture for another 2-3 days in G2 culture medium (G2, VitroLife, Gothenburg, Sweden).

Frozen-thawed

Cleavage embryos and blastocysts were vitrified and warmed using Kitazato media (Kitazato, Tokyo, Japan) and Fibreplugs (CVM kit, Cryologic, Melbourne, VIC, Australia). The protocol for freezing-thawing is based on the Cryotop method, abiding by the standard protocol. Only grade 1 and 2 embryos on day 3 and grade A and B blastocyst on day 5 and day 6 were frozen.[8] The embryos were warmed for 2 hours and cultured in suitable media under mineral oil at 37℃ in 5% CO2 before being transferred. A maximum of three surviving embryos,

after thawing, were transferred into the uterus.

Luteal support and pregnancy

The luteal phase was assisted with Estradiol Valerate (Progynova, Bayer, Indonesia) orally at the dose of 6 mg every day starting from the second day of the menstrual cycle. The patients were administrated with Cyclogest Ovaginal pessaries (Cox Pharmaceuticals, Barnstaple, UK) 400 mg twice daily when the endometrial thickness reached more than 8 mm and continued until twelve weeks of gestation.

The outcomes measured were the number of embryos recovered after freezing-thawing, chemical pregnancy rate (level of hCG ≥50 mIU/ml), the implantation rate (the ratio of gestational sac/number of embryos transferred), and live birth rate (number of baby delivery/number of gestational sac), miscarriage, anembryonic pregnancy and IUFD pregnancy rate.

STATISTICAL ANALYSIS

The data were expressed as mean ± SD or number (percentage) and analyzed using the SPSS version 26.0. Means were analyzed using ANOVA for parametric data followed by Bonferroni if the data were normally distributed, or Games-Howell test if the data were not normally distributed. Kruskal-Wallis test was used for non-parametric data. Proportions were compared using Chi-Squared test. All statistical significance was set as $P<0.05$.

RESULTS

1. Basic Characteristics

The results of the study showed that the number of samples in each group in the period of March 2018 – December 2022 was greater in individuals with frozen embryo transfer on the 5 compared to day 3 and day 6 (Table 1). There were no differences in age among samples, with the average age of the entire group approximately 33 years old.

Table 1. Characteristic of the Research Sample in Each Group

Characteristics	Frozen Embryo Transfer Day-3	Frozen Embryo Transfer Day-5	Frozen Embryo Transfer Day-6
Number of patients [n]	29	110	18
Age (Years)	33.38 ± 4.14	32.38 ± 3.84	34.56 ± 3.50

2. Embryo Quality

Based on the category, the number of embryos thawed per patient on day 3 was the highest on average compared to day 5 and day 6. There was no significant difference in numbers of embryos recovered post freezing-thawing among groups. The number of embryos transferred on day 3 was significantly higher compared to day 5 and day 6 frozen embryo transfers (**Table 2).**

Table 2. Comparison of Embryo Quality in Each Group

	Frozen Embryo Transfer Day-3	Frozen Embryo Transfer Day-5	Frozen Embryo Transfer Day-6
Total number of embryos freezing-thawing	65	203	30
Number of Embryos thawed per patients	2.24 ± 1.023[a]	1.83 ± 0.812[b]	1.67 ± 0.686[b]
Number of Embryos recovered After warming (*survival rate*)	2.10 ± 0.976	1.79 ± 0.796	1.56 ± 0.616
Number of Dead Embryos After warming	0.14 ± 0.441	0.04 ± 0.186	0.11 ± 0.471
Number of Embryos Transferred	2.07 ± 0.884[a]	1.56 ± 0.499[b]	1.44 ± 0.511[b]

Different letters in columns indicate significant differences in the statistical test ($P<0.05$)

3. Success Rates

The pregnancy and implantation rates were significantly higher on day 5 frozen embryo transfer compared to day 3 and day 6 frozen embryo transfers. There was no difference in pregnancy and implantation rates between day 3 and day 6 frozen embryo transfer groups. Live birth rates were similar among

groups. One woman on day 3 and day 6 frozen embryo transfer groups and 9 women on day 5 frozen embryo transfer suffered from anembryonic pregnancy. Only one woman from day 5 frozen embryo transfer suffered from IUFD.

Table 3. Comparison of the Success Rates of the IVF Program in Each Group

	Frozen Embryo Transfer Day-3	Frozen Embryo Transfer Day-5	Frozen Embryo Transfer Day-6
Pregnancy Rates	10/29 (34.5%)[a]	59/110 (53.6%)[b]	6/18 (33.3%)[a]
Implantation Rates	10/59 (11.9%)[a]	62/172 (36.0%)[b]	6/26 (19.2%)[a]
Miscarriage Rates	1/10 (10%)	8/62 (12.9%)	0
IUFD *	0	1	0
Anembryonic Pregnancy Rates*	1/10 (10.0%)	9/62 (14.5%)	1/6 (16.7%)
Live Birth Rates*	8/10 (80.0%)	44/62 (70.9%)	5/6 (83.3%)

Different letters in columns indicate different meanings in statistical tests (P<0.05)
*the number is too small for statistical analysis.

DISCUSSION

The results of the pregnancy and implantation rates in this present study were significantly higher for the day 5 blastocyst embryo transfers, 53.6% and 36% respectively, when compared to the day 3 cleavage and day 6 blastocyst frozen embryo transfers. These results support the benefits of expanding embryo culture to the blastocyst stage in order to influence an increase in implantation and pregnancy rates.[11,12] Similar results were obtained from research conducted by Eftekhar *et al* (2020) showing blastocyst frozen embryo transfer had significantly increased pregnancy and implantation rates compared to cleavage embryo transfer [12]. Another study showed that day 3 frozen embryo transfer with high quality, determined using time-lapse monitoring, compared to blastocyst-stage embryos, assessed through conventional morphological selection, obtained the highest implantation rate in blastocyst-stage embryos.[13] The higher pregnancy rate can be explained because blastocyst culture can help improve embryo screening to select the best quality embryos.[14] These results indicate that blastocyst culture is a more effective method to select embryos because they have greater potential for development. Interestingly, our results showed that the pregnancy and implantation rates from day 6 frozen blastocyst transfer was significantly lower compared to day 5 frozen blastocyst transfer. These results are consistent with previous results.[15,16,17,18] Previous research has shown that the expansion stage is an important factor in embryos undergoing implantation, so assessing the quality of trophectoderm and inner cell mass at the blastocyst stage is very important.[19] The implantation rate for day 6 frozen embryo transfer was much lower than for day 5 and could be due to the slow rate of development into a blastocyst (late blastocyst), and the possibility of increased DNA damage leading to aneuploidy and resulting in a lower implantation rate.[18,20]

Miscarriage rate is classified as the number of pregnancy losses in the 20 weeks before clinical pregnancy. There was no significant difference in the miscarriage rate between the 3 frozen embryo transfer groups. The day 3 group obtained a miscarriage rate of 10% and the day 5 group obtained 12.9%. Meanwhile, there was no miscarriage rate with the day 6 frozen embryo transfer group. Gao *et al* (2023) and Rao *et al* (2021) showed similar results regarding the miscarriage rate which were also not significantly different between the three groups.[2,18] In this study, no incidence of ectopic pregnancy was found in any frozen embryo transfer group, but based on research conducted by Rao *et al* (2021), the rate of ectopic pregnancy in the three groups did not have a significant difference. A previous study showed the occurrence of ectopic pregnancies on day 3, 5 and 6 frozen embryo transfers to be small.[18] The rate of anembryonic pregnancy in the three groups

was not significantly different and was highest on day 6 frozen embryo transfer.

In this present study, there were no significant differences in living birth rate among the three groups. A previous study reported that there were no differences in ongoing pregnancies and live birth rates between cleavage or blastocyst stage frozen embryo transfers.[21] However, research conducted by Eftekhar *et al* (2020) showed that live birth rate was significantly higher in the blastocyst compared to cleavage frozen embryo transfer groups.[14] Interestingly, Rao *et al* (2021) found that there were no significant differences in the biochemical pregnancy rate, clinical pregnancy rate, or ongoing pregnancy rate between the day 3 and day 5 frozen embryo transfer groups but these rates were significantly higher when compared with those from the day 6 frozen embryo transfer group.

The benefits of frozen embryo transfer carried out based on the three groups can be influenced by other important factors, such as the grade of the embryo, the number of embryos transferred, and the age of the mother both at the time of egg induction and when the frozen embryo transfer is carried out, with an increase in maternal age associated with decreasing quality of the oocytes.[22,23]

CONCLUSION

In this present study, we found that there were no differences in recovery rates of cleavage and day 5 and day 6 blastocysts stages after frozen-thawed. However, pregnancy and implantation rates were significantly higher in day 5 frozen embryo transfer compared to those on day 3 and day 6 frozen embryo transfers. No significant differences were found in miscarriage rate, IUFD, and live birth rate in the three groups.

Declaration by Authors

Ethical Approval: This research was approved by the Ethics Committee of the Faculty of Medicine Udayana University (No: 2455/UN14.2.2.VII.14/LT/2023).

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