Analysis of day 3 and day 5 embryo transfer in fresh and frozen cycles to assess in vitro fertilization success

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Abstract

The objective was to compare embryo quality and pregnancy rates associated with fresh and frozen embryo transfer (ET) protocols. The study examined 697 patients who underwent IVF using either fresh ET cycles or frozen embryo transfer (FET) cycles. A retrospective study of laboratory data from January 2018 through December 2022 was investigated. Out of the total 361 patients who underwent IVF with fresh ET cycles, 241 had Day 3 transfers, and 120 had Day 5 transfers. Among the 336 patients who underwent frozen FET, 119 had Day 3 transfers, and 217 had Day 5 transfers. The investigation revealed a disparity in the quality (grade) of embryos on Day 3 and Day 5 during fresh cycles (mean grades 3.0 vs 3.3 respectively, p=0.002), while a similar distinction was not observed in frozen cycles (mean grade 2.9 vs 3.0 respectively, p=0.205). In fresh cycles, there was no substantial contrast in pregnancy rates between embryos transferred on Day 3 and Day 5 (38.2% vs 40.0%, p=0.150); however, in frozen cycles, the difference was statistically significant (32.8% vs. 47.5%, p=0.026). Implantation rates for embryos transferred on Day 3 and Day 5 in fresh (31.5% vs 35.8%, p=0.086) and for frozen embryos (26.1% vs. 41.5%, p=0.116) were not statistically significant. Fresh and frozen embryo transfers on Day 3 and Day 5 are equally effective. There is no significant difference in embryo quality, pregnancy, and implantation rates between the two cycles, except for Day 5 embryo quality (p=0.004). In summary, our study demonstrates that fresh ET and FET on Day 3 and Day 5 exhibit comparable effectiveness in terms of embryo quality, pregnancy rates, and implantation rates. While a significant difference in Day 5 embryo quality was noted, this finding emphasizes the overall equality in outcomes between the two cycles, providing valuable insights for informed ART decision-making.

Disclaimer: The authors declare no conflict of interest.
J Reprod Biotechnol Biomed Sci.14:29-34
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Compliance acknowledgement: This article was edited by the Australian Editorial Services (www.nativeenglisheditor.com)
Keywords: Embryo transfer, frozen embryo transfer, cleavage stage, blastocyst stage, embryo quality
Notification: The statements and claims in this manuscript are that of the author(s) alone.

Introduction

In assisted reproductive techniques (ART), embryo culture and transfer are crucial steps in establishing a successful pregnancy. The optimization of these processes has been a subject of ongoing debate and research within the field of reproductive medicine. One key aspect under scrutiny is the quality of embryos transferred on days 3 and 5 during both fresh and frozen IVF cycles. Embryo quality can influence pregnancy success rates substantially. While some studies suggest higher pregnancy rates in frozen embryo transfer (FET) cycles compared to fresh cycles (Bushaqer et al., 2020), the choice of culture medium also plays a role in the quality of embryos generated (Tao et al., 2013). However, controversies persist

regarding the optimal timing for embryo transfer, whether at the cleavage or blastocyst stage. While day 5 transfers may require fewer embryos, their superiority in terms of clinical pregnancy, implantation, and live birth rates compared to day 3 transfers remains debatable (Garbhini, 2022).

Furthermore, a key determinant of embryo transfer success is embryo quality, which is evaluated using standardized grading systems. Cleavage-stage embryos are graded based on cell number, fragmentation, and symmetry (Ali et al., 2000), while blastocyst-stage embryos are assessed according to morphological parameters and numerical scoring methods designed to improve reproducibility and predictive value (Ali, 2014; Ali et al., 2025; Al Helou, 2025). These grading systems provide critical guidance in embryo selection, yet the ability to consistently identify the most viable embryos remains a challenge in clinical practice.

The transition from cleavage-stage to blastocyst-stage embryo transfer represents a significant development in ART, with blastocyst culture showing promise in improving implantation rates and pregnancy outcomes (Le et. al., 2018). However, concerns regarding potential adverse perinatal outcomes associated with extended culture to the blastocyst stage necessitate a comprehensive understanding of its implications. Studies have highlighted the impact of blastocyst culture on reducing the need for multiple embryo transfers (Gardner, 2000), but questions remain regarding its association with preterm delivery and congenital anomalies. Consequently, there is a need for further investigation to elucidate the optimal approach for embryo selection and transfer.

Building upon existing literature, this study aims to analyse whether blastocyst-stage embryo transfer enhances implantation and pregnancy rates compared to cleavage-stage embryo transfer in Malaysian women undergoing IVF treatment, considering variations in embryo transfer cycles. By comparing day 3 (cleavage stage) and day 5 (blastocyst stage) embryo transfers in both fresh and frozen cycles, this research seeks to contribute to the ongoing discourse surrounding optimal embryo transfer practices in ART.

Methodology

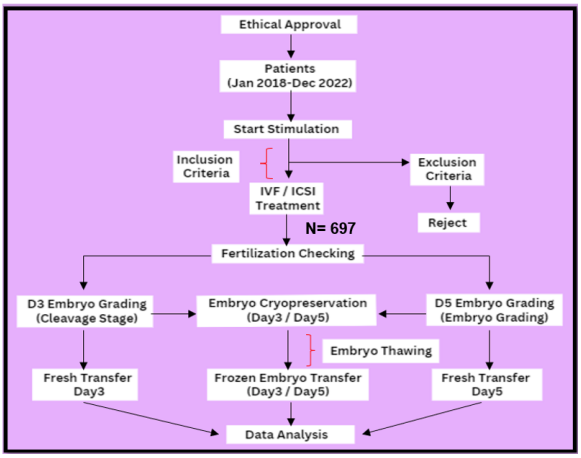

Figure 1 Study Flowchart

This retrospective investigation compares the impact of different embryo transfer cycles on the quality of embryos in IVF procedures. Embryo transfers were conducted on both days 3 and 5 in fresh cycles, while the remaining embryos were cultured and cryopreserved at the cleavage and blastocyst stages for frozen embryo transfer (FET). The grading system used for embryos ranged from excellent to poor numerically in a range of 4 to 1 for cleavage stage embryos (Ali et al., 2000) and blastocysts (Ali, 2014; Ali et al., 2025).

The study utilized secondary data from patient case notes at the Embryology Laboratory of the UMMC in Kuala Lumpur, Malaysia, covering the period from January 2018 to December 2022. A total of 697 IVF patients were included in the analysis, with 361 undergoing fresh embryo transfer (ET) cycles and 336 undergoing FET cycles. Among these, 241 patients underwent day 3 transfers, and 120 underwent day 5 transfers in fresh cycles, while 119 patients had day 3 transfers and 217 had day 5 transfers in FET cycles.

Sample size determination was conducted using an online analyzer (http://clincalc.com/Stats/SampleSize.aspx) before the study. Data were entered into a customized database and analyzed using IBM Statistical Package for Social Science (SPSS) version 21. Chi-squared tests were employed for

categorical variables, while Paired-Samples T-tests were used for continuous variables when comparing data groups. Statistical significance was defined as a p-value of less than 0.05.

Results

Six hundred and ninety-seven patients (n=697) met the inclusion criteria, with 361 (51.8%) patients having fresh ET and 336 (48.2%) patients undergoing FET. Patients over 42 years of age were excluded from this study because the patient's age of ≥42 had passed the acceptance criteria at UMMC for fertility treatment. ET was carried out in all patients either at the cleavage stage on day 3 or at the blastocyst stage on day 5.

Patient demographics and characteristics

Fresh cycle of embryo transfer

A total of 361 patients underwent fresh embryo transfer, out of which 241 (66.8%) patients had their transfer on Day 3 and 120 (33.2%) patients had it on Day 5. Clinical characteristics of the fresh embryo transfers on Day 3 and Day 5 are shown in Table 1. There were no significant differences in the age and ethnicity of women between the two groups (p=0.223 and p=0.239, respectively). However, there were significant differences in the selection of the treatment type in Day 3 compared to Day 5 (1.8±0.7 vs. 1.5±0.7; p=0.008).

Table 1 Patient demographics (Fresh Embryo Transfer)

Indexes	Overall	Day 3 mean±SD	Day 5 mean±SD	*P* Value
Number of patients (n)	361	241(66.8%)	120(33.2%)	
Age of female		2.2±0.6	2.1±0.5	0.223
• <30		24 (10.0%)	13 (10.8%)	
• 31-37		142 (58.9%)	85 (70.8%)	
• 38>		75 (31.1%)	22 (18.4%)	
Ethnicity		1.5±0.8	1.4±0.8	0.239
• Malay		170 (70.5%)	89 (74.2%)	
• Chinese		37 (15.4%)	19 (15.8%)	
• Indian		26 (10.8%)	8 (6.7%)	
• Others		8 (3.3%)	4 (3.3%)	
Type of ART		1.8±0.7	1.5±0.7	**0.008***
• IVF		104 (43.2%)	76 (63.3%)	
• ICSI		108 (44.8%)	27 (22.5%)	
• IVF + ICSI		29 (12.0%)	17 (14.2%)	

*p<0.05 was accepted as statistically significant

Table 2 presents there were significantly more high-quality embryos in Day 5 transfers compared to Day 3 transfers (3.3±0.6 vs. 3.0±0.8; p=0.002).

Table 2 Quality of Embryo Day 3 and Day 5

Indexes	Overall n=361	Day 3 mean±SD	Day 5 mean±SD	*P* Value
Embryo Grade		3.0±0.8	3.3±0.6	**0.002***
Cleavage Stage				
• 1-2 (poor)		43 (17.8%)		
• >2-4 (good)		198 (82.2%)		
Blastocyst Stage				
• 1-2 (poor)			6 (5.0%)	
• >2-4 (good)			114 (95.0%)	

*p<0.05 was accepted as statistically significant

Frozen embryo transfer

A total of 336 patients underwent Frozen Embryo Transfer (FET), out of which 119 (35.4%) had the transfer on day 3 FET, while 217 (64.6%) had it on day 5 FET as demonstrated in Table 3. In the clinical characteristics of patients who had FET on day 3 and day 5, it was found that there were no significant differences in both the day 3 and day 5 FET transfer groups in terms of ethnicity and type of treatment. However, there were significant differences in the age of females in the day 3 FET group compared to the day 5 FET group (1.2±0.6; 1.0±0.6; p=0.015) (Table 3).

Table 3 Patient Demographics (Frozen Embryo Transfer)

Indexes	Overall	Day 3 mean±SD	Day 5 mean±SD	*P* Value
Number of patients (n)	336	119(35.4%)	217(64.6%)	-
Age of female		1.2±0.6	1.0±0.6	**0.015***
• <30		10 (8.4%)	28 (12.9%)	
• 31-37		75 (63.0%)	148 (68.2%)	
• 38>		34 (28.6%)	41 (18.9%)	
Ethnicity (mean/SE)		0.3±0.7	0.5±0.8	0.191
• Malay		92 (77.3%)	154 (71.0%)	
• Chinese		14 (11.8%)	31 (14.3%)	
• Indian		13 (10.9%)	32 (14.8%)	
Type of ART		0.6±0.6	0.5±0.8	0.923
• IVF		62 (52.1%)	131 (60.4%)	
• ICSI		48 (40.3%)	46 (21.2%)	
• IVF + ICSI		9 (7.6%)	40 (18.4%)	

*p<0.05 was accepted as statistically significant

Table 4 presents there were no significant differences in the quality of embryo grades between Day 3 and Day 5 FET (2.9±0.8; 3.0±0.9; P=0.205).

Table 4 Patient Demographics (Frozen Embryo Transfer)

Indexes	Overall n=336	Day 3 mean±SD	Day 5 mean±SD	*P* Value
Embryo Grade		2.9±0.8	3.0±0.8	0.205
Cleavage Stage				
• 1-2		28 (23.5%)		
• >2-4		91 (76.5%)		
Blastocyst Stage				
• 1-2			28 (12.9%)	
• >2-4			189 (87.1%)	

*p<0.05 was accepted as statistically significant

Pregnancy outcomes and embryo quality (Fresh cycle)

The results of a study comparing the outcomes of day 3 and day 5 embryo transfer (ET) in a fresh cycle are summarized in Table 3. The clinical pregnancy and implantation rates for both ET types were found statistically similar (38.2% vs 40.0%; p=0.150 and 31.5% vs 35.8%; p=0.086, respectively). However, the miscarriage rate was significantly higher for day 3 ET compared to day 5 ET (6.6% vs 4.2%; p=0.001). Additionally, the embryo quality was also found to be significantly lower for day 3 ET compared to day 5 ET (3.0±0.8 vs 3.3±0.6; p=0.002, respectively) (Table 5).

Table 5 Pregnancy Outcomes and Embryo Quality (Fresh Cycle)

Indexes	Overall	Day 3 mean±SD	Day 5 mean±SD	*P* Value
Number of patients (n)	361	241(66.8%)	120(33.2%)	
Pregnancy rate		92(38.2%)	48(40.0%)	0.150
Implantation rate		76(31.5%)	43(35.8%)	0.086
Miscarriage rate		16(6.6%)	5(4.2%)	**0.001***
Embryo quality		3.0±0.8	3.3±0.6	**0.002***

*p<0.05 was accepted as statistically significant

Pregnancy outcomes and embryo quality (frozen embryo transfer)

The outcomes of a study that compared day 3 and day 5 embryo transfer (ET) in a frozen cycle are presented in Table 6. The study found that there was no significant difference in the implantation rate and embryo quality for both types of FET. The implantation rates were 26.1% vs 41.5% with p=0.116, and embryo quality was 2.9±0.8 vs 3.0±0.8 with p=0.205, respectively. However, the study found that the pregnancy rate was significantly higher for day 5 FET compared to day 3 FET (47.5% vs 32.8%; p=0.026). Additionally, the study found that the miscarriage rate was significantly higher for day 3 FET compared to day 5 FET (6.7% vs 6.0%, p= 0.025 respectively).

Table 6 Pregnancy Outcomes and Embryo Quality (Frozen Embryo Transfer)

Indexes	Overall	Day 3 mean±SD	Day 5 mean±SD	*P* Value
Number of patients (n)	336	119(35.4%)	217(64.6%)	
Pregnancy rate		39(32.8%)	103(47.5%)	**0.026***
Implantation rate		31(26.1%)	90(41.5%)	0.116
Miscarriage rate		8(6.7%)	13(6.0%)	**0.025***
Embryo quality		2.9±0.8	3.0±0.8	0.205

*p<0.05 was accepted as statistically significant

Day 3 fresh embryo transfer vs day 3 frozen embryo transfer

Table 7 presents the comparison between two groups of embryo transfer (ET) concerning the ET day on day 3. The statistical analysis of the data indicated no significant differences in pregnancy rate, implantation rate, and embryo quality in both cycle transfers (p=0.897, p=0.889, p=0.308 respectively). However, the data showed an increase in miscarriage rate with day 3 fresh ET versus day 3 FET (0.1±0.3 vs 0.1±0.3; p=0.004).

Table 7 Comparison of Day 3 ET vs Day 3 FET

Indexes	Overall	Day 3 (ET) (mean/SD)	Day 3 (FET) (mean/SD)	*P* Value
Number of patients (n)	360	241(66.9%)	119(33.1%)	
Pregnancy rate		0.7±0.5	0.7±0.5	0.897
Implantation rate		0.7±0.5	0.7±0.4	0.889
Miscarriage rate		0.1±0.3	0.1±0.3	**0.004***
Embryo quality		3.0±0.8	2.9±0.8	0.308

*p<0.05 was accepted as statistically significant

Day 5 fresh embryo transfer vs day 5 frozen embryo transfer

Table 8 presents a comparison between two groups of embryo transfers (ET) based on the day of ET, either on day 5 fresh ET or day 5 FET. According to the statistical analysis of the data, there were no significant differences in the pregnancy rate and implantation rate for both cycles. The pregnancy rates respectively were 0.6±0.5 vs 0.5±0.5 with p=0.278, and implantation rates were 0.6±0.5 vs 0.7±0.5 with p=0.887. However, the data shows that the miscarriage rate was significantly higher on day 5 FET compared to Day 5 ET (0.1±0.3 vs 0.04±0.2; p=0.004, respectively). Additionally, the quality of embryos resulted in significantly higher rates on day 5 fresh ET (3.3±0.6 vs 3.0±0.8; p=0.004, respectively) compared to day 5 FET.

Table 8 Comparison of Day 5 ET vs Day 5 FET

Indexes	Overall	Day 5 (ET) (mean/SD)	Day 5 (FET) (mean/SD)	*P* Value
Number of patients (n)	337	120(35.6%)	217(64.4%)	
Pregnancy rate		0.6±0.5	0.5±0.5	0.278
Implantation rate		0.6±0.5	0.7±0.5	0.887
Miscarriage rate		0.04±0.2	0.1±0.3	**0.004***
Embryo quality		3.3±0.6	3.0±0.8	**0.004***

*p<0.05 was accepted as statistically significant

Discussion

Fresh embryo transfer cycle

The data indicated no significant differences in age and ethnicity between day 3 and day 5 transfers. However, our study showed a significantly higher number of high-quality embryos in day 5 transfers, aligning with findings from Kaur et. al., (2014), who reported elevated

implantation and clinical pregnancy rates with day 5 transfers. Our findings showed statistically similar clinical pregnancy and implantation rates between day 3 and day 5 transfers, aligning with previous studies by Hatırnaz (2017) and Alfaraj et. al., (2017). However, Day 3 transfers exhibited a significantly higher miscarriage rate and lower embryo quality in our dataset.

Frozen Embryo Transfer Cycle

No significant differences existed between day 3 and day 5 groups in ethnicity, treatment type, and embryo grade. Studies, including Rao et. al., (2021) and Alfaraj et. al., (2017), affirm that pregnancy rates, implantation rates, and clinical outcomes are comparable between day 3 and day 5 Frozen Embryo Transfer (FET). Considering morphological grading, higher grades may positively impact pregnancy outcomes (Guo, 2019). Our data indicated no significant differences in implantation rates and embryo quality. Yet, day 5 FET exhibited a significantly higher pregnancy rate and lower miscarriage rate compared to day 3 FET. This aligns with Liu et. al., (2014) findings, highlighting the superior implantation and clinical pregnancy rates in frozen-thawed cycles compared to fresh cycles, with no disparity in miscarriage rates.

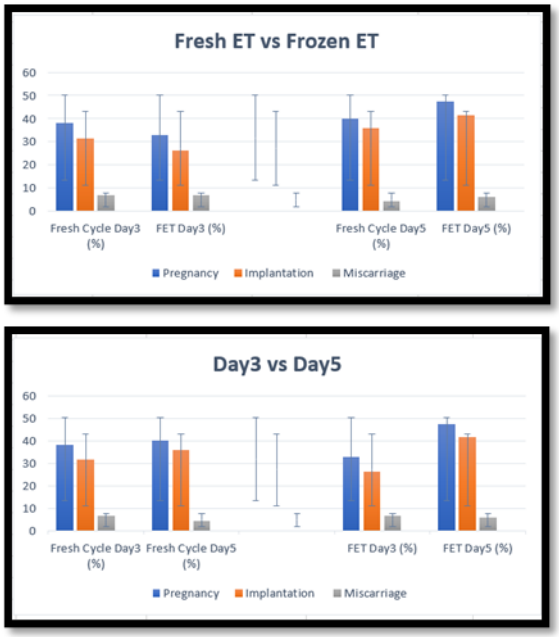

Figure 2 Comparison of pregnancy, implantation and miscarriage rates between Day 3 and Day 5 embryo transfer in different cycles (Fresh ET vs FET)

In summary (Figure 3), our study revealed significantly higher miscarriage rates in both transfer cycles. Maternal demographics, including endometrial pattern, thickness, and age, were found to be highly significant predictors of embryonic implantation in IVF (Ugwu et. al., 2021). For day 3 transfers, no significant differences were noted in pregnancy, implantation rates, or embryo quality between fresh and frozen cycles. However, fresh day 3 transfers exhibited a higher miscarriage rate compared to day 3 FET. Similarly, day 5 transfers in fresh and frozen cycles showed no significant differences in pregnancy or implantation rates. Yet, fresh day 5 transfers displayed superior embryo quality and a lower miscarriage rate compared to day 5 FET. Research by Mohamed et. al., (2019) suggests that maintaining embryo culture until day 5 in frozen embryo transfers may be more effective in identifying high-quality embryos compared to transfers on day 3.

Parameters	Fresh Cycle Day3 (%)	Fresh Cycle Day5 (%)	P Value (P<0.05)
Pregnancy	38.2	40.0	0.15
Implantation	31.5	35.8	0.086
Miscarriage	6.6	4.2	0.001
Embryo Quality (mean/SD)	3.0 ± 0.8	3.3 ± 0.6	0.002
	FET Day3	FET Day5	
Pregnancy	32.8	47.5	0.026
Implantation	26.1	41.5	0.116
Miscarriage	6.7	6.0	0.025
Embryo Quality (mean/SD)	2.9 ± 0.8	3.0 ± 0.8	0.205
	Fresh Cycle Day3	FET Day3	
Pregnancy	38.2	32.8	0.897
Implantation	31.5	26.1	0.889
Miscarriage	6.6	6.7	0.004
Embryo Quality (mean/SD)	3.0 ± 0.8	2.9 ± 0.8	0.308
	Fresh Cycle Day5	FET Day5	
Pregnancy	40.0	47.5	0.278
Implantation	35.8	41.5	0.887
Miscarriage	4.2	6.0	0.004
Embryo Quality (mean/SD)	3.3 ± 0.6	3.0 ± 0.8	0.004

Figure 3 Summary result of the study

Conclusion

Our study demonstrates that fresh ET and FET on Day 3 and Day 5 exhibit comparable effectiveness in embryo quality, pregnancy rates, and implantation rates. While a significant difference in Day 5 embryo quality was noted, this finding emphasizes the overall equality in outcomes between the two cycles, providing valuable insights for informed ART decision-making.

Acknowledgement

I would like to express my sincerest gratitude to Madam Sharifah Izwan Tuan Othman and Madam Siti Khadijah Idris as supervisor and co-supervisor respectively. Special thanks to the staff of the Embryology Laboratory at UMMC and Universiti Selangor for their guidance, expertise, and unwavering support throughout this research.

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