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A retrospective comparative study of double cleavage-stage embryo transfer versus single blastocyst in frozen-thawed cycles

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Abstract

Background This retrospective study aimed to compare the outcomes of day 3 double embryo transfer (DET) with single blastocyst transfer (SBT) during frozen embryo transfer (FET) cycles. A total of 999 women below the age of 38 years who underwent FET at Malaysia's KL Fertility and Gynaecology Centre from January 2019 to December 2021 were analyzed. Patients with autologous eggs were recruited in the study. All the eggs were inseminated by intracytoplasmic sperm injection. The embryos were vitrified on day 3 cleavage-stage or blastocyst stage with Cryotop® method. The FET was performed following natural cycle (NC), modified natural cycle (m-NC), or hormone replacement therapy (HRT) cycles. The NC and m-NC groups received oral dydrogesterone for luteal phase support.

Results There were no statistical differences in the rates of positive pregnancy, clinical pregnancy, and ongoing pregnancy between the two groups. However, implantation rates were significantly higher in the SBT group (50.1% versus 37.6%, $p < 0.05$). The day 3 DET group had significantly higher multiple pregnancy rates (28.7% versus 1.1%, $p < 0.05$). Subgroup analysis of embryo transfers performed following NC, m-NC, or HRT cycles showed similar results.

Conclusions This study suggests that SBT is the better choice for embryo transfers as it had higher implantation rates and its pregnancy rates were similar to day 3 DET. The SBT also significantly reduced the incidence of multiple pregnancies without compromising pregnancy rates.

Keywords Frozen embryo transfer, Positive pregnancy rate, Clinical pregnancy rate, Ongoing pregnancy rate, Multiple pregnancy rate, Implantation rate

Background

Although multiple embryo transfers can increase the likelihood of live births for women undergoing in vitro fertilization (IVF), multiple pregnancies, maternal complications, and neonatal complications can occur [1]. Women with multiple pregnancies are more likely to suffer miscarriages, fetal deaths, fetal malformations, and pregnancy complications than single pregnancies [2]. Multiple pregnancies have resulted from difficulty in selecting the most competent embryos on day 2/3 for transfer during IVF treatment, and hence transferring more than one embryo to ensure higher pregnancy rates [3].

The approach of blastocyst transfers has gained traction due to the reduced risk of multiple pregnancies and improved clinical outcomes than the use of embryos at earlier embryonic development stages (cleavage embryo transfer) [2, 4–7]. However, at the time of writing this paper, there are no guidelines or consensus on blastocyst and cleavage-stage transfers. There is a 2022 consensus on embryo transfer, but the publication was focused on technical aspects before, during, and after the procedure [8]. Several studies have examined the clinical outcomes

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of the blastocyst-stage transfer but a 2022 Cochrane review concluded that more evidence was needed to determine the impact of the stage of transfer on cumulative live births and pregnancy rates [9]. Our objective in the present study was to assess the success rates between day 3 double cleavage-stage embryo transfer (DET) and single blastocyst transfer (SBT) in women undergoing frozen embryo transfer (FET) with three different endometrial preparation methods, i.e., natural cycle (NC), modified natural cycle (m-NC), and hormone replacement therapy (HRT) in a single fertility center in Malaysia.

Methods

Subjects

This retrospective study included data from 999 female patients below the age of 38 years who underwent day 3 DET or SBT during FET cycles at the KL Fertility and Gynaecology Centre, Malaysia from January 2019 to December 2021. Patients with primary and secondary subfertility, male factors, tubal factors, polycystic ovary syndrome, and endometriosis were included. Exclusion criteria were gamete donation, embryo donation, preimplantation genetic testing, assisted hatching, and cases with incomplete information such as overseas patients or patients referred from other centers.

All the patients' details were deidentified.

Culture of embryos

All the eggs were inseminated by intracytoplasmic sperm injection (ICSI) at 38 to 40 h after human chorionic gonadotrophin (hCG) administration. The day of insemination was defined as day 0. Embryos were examined for fertilization on day 1 (16–18 h post-ICSI), and subsequently examined on day 3 and day 5/6/7 for embryo grading. Two day 3 embryos of high-quality grade 1 or 2 were vitrified as per the center's protocol, and the rest were cultured further to day 5/6/7. For the blastocyst stage, inner cell mass (ICM) and trophectoderm of grades BC, CB, or above were selected for vitrification. Embryo culture was performed at 37 °C, under 6% CO_2, 5% O_2, and 89% N_2 using sequential culture media (COOK, Sydney IVF) in the benchtop incubator (Origio/Planer BT37).

Embryo grading

Grade 1 day 3 cleavage-stage embryo consisted of (1) 6–10 cells, (2) even-sized blastomeres, and (3) no fragmentation. Grade 2 day 3 cleavage-stage embryo consisted of (1) 6–8 cells, (2) slightly uneven-sized blastomeres, and (3) fragmentation < 10%. Grade 3 cleavage-stage embryo consisted of (1) < 6 cells, (2) uneven-sized blastomeres, and (3) 10–25% fragmentation. And Grade 4 cleavage-stage embryo consisted of (1) < 4 cells, (2) uneven-sized blastomeres, and (3) > 25% fragmentation. For day 3 DET, embryos with grade 1 and grade 2 were chosen for vitrification and transfer.

For day 5/6/7 blastocyst grading, inner cell mass, and trophectoderm were graded as A, B, C, and D respectively. Grade A is excellent, grade B is good, grade C is average and grade D is poor. Full blastocyst, expanded blastocyst, hatching blastocyst, or fully hatched blastocyst with ICM and trophectoderm of grades A, B, or C were selected for vitrification and embryo transfer.

Vitrification and thawing of cleavage-stage embryos and blastocysts

The cleavage-stage embryos and blastocysts were vitrified and thawed with Kitazato vitrification media and Kitazato thawing media (Kitazato Corporation, Japan) as described by Kuwayama (2007) [10] using the Cryotop method.

The cleavage-stage embryos were exposed to the equilibrium solution at room temperature for 12 min while the blastocyst was exposed for 15 min before being transferred into the vitrification solution for dehydration for 1 min. The embryos were subsequently loaded into the Cryotop. The carrier was directly plunged into liquid nitrogen.

The cleavage-stage embryo and blastocyst underwent the same thawing procedure. During thawing, the Cryotop is quickly immersed into the thawing solution (at 37 °C, for 1 min). Next, the embryos were transferred into dilution solution for 3 min followed by washing solution 1 (for 5 min) and washing solution 2 (for 1 min). Finally, the embryos were transferred to the blastocyst medium (COOK, Sydney IVF) for further culture until embryo transfer.

Frozen embryo transfer

For endometrial preparation, the endometrium was adjusted to the cleavage stage or the transfer window of blastocysts.

The FET was performed following natural cycle (NC), modified natural cycle (m-NC), or hormone replacement therapy (HRT) cycles. Patients who underwent NC cycles were scanned at day 2–3 menses to exclude cysts or prevailing corpus luteum from the previous cycle. Transvaginal ultrasound scans were carried out again on days 10–12 to determine the size of the dominant follicle. Once the dominant follicle attained a size of 16 mm or above, daily self-testing of urine luteinizing hormone (LH) was done to ascertain the timing of ovulation. Embryo transfers were carried out 4 or 6 days after a positive LH test. Oral dydrogesterone 10 mg twice daily was

given as luteal phase support (LPS) until tested positive for pregnancy and extended to 12 weeks of gestation.

The initial monitoring for m-NC was similar to that for NC and patients were scanned by transvaginal ultrasound on days 10–12 of the menstrual cycle to determine the size of the dominant follicles. Ovulation was triggered with 5000 i.u hCG when the dominant follicle reached a mean diameter of 16 mm. Embryo transfers were carried out 4 or 6 days after ovulation induction. Oral dydrogesterone 10 mg twice daily was given as LPS.

Patients who underwent HRT cycles were scanned at day 2–3 menses and started on a fixed constant dose of oral estradiol valerate 6 mg daily (Progynova 2 mg × 3 daily) for 10–12 days. A transvaginal ultrasound was performed after 10–12 days of estradiol administration to measure endometrial thickness and confirm the absence of leading follicles. When the endometrial thickness reached 7 mm and above, vaginal micronized progesterone supplementation (Utrogestan 200 mg × 3 times daily) was commenced and FET was scheduled 4 or 6 days later. The hormonal treatment was continued until 12 weeks in the event of pregnancy, which was determined by serum measurement of hCG 10–12 days after transfer.

For women with cleavage embryos and blastocysts, two day 3 cleavage-stage embryos were transferred. If there was no pregnancy, a single blastocyst was used. For women who only had one day 3 cleavage-stage embryo and blastocysts, a single blastocyst was used first.

Outcome measures

Positive pregnancy was defined as elevated hCG concentration > 25 IU/L on day 10 or day 12 post-transfer respectively. The rate of positive pregnancy was calculated as the percentage of positive pregnancies over the total number of transfer cycles.

Clinical pregnancy was defined as evidenced by transvaginal ultrasound of an intrauterine sac at 5 weeks after transfer (with or without a fetal heart). The rate of clinical pregnancy was calculated as a percentage of clinical pregnancy over the total number of transfer cycles.

Implantation was defined as implanted embryos at six weeks. The rate of implantation was calculated as a percentage of implanted embryos at six weeks over the total of transferred embryos.

Multiple pregnancies were defined as the presence of two or more gestational sacs at 12 weeks. The rate of multiple pregnancies was calculated as a percentage of twin pregnancies over the total of clinical pregnancies.

Ongoing pregnancy was defined as a pregnancy that completed 12 weeks of gestation. The rate of ongoing pregnancy was calculated as a percentage of ongoing pregnancies over the total of transfer cycles.

Table 1 Baseline characteristics for day 3 double cleavage-stage transfer group versus single blastocyst transfer group

	DET day 3	SBT	*p* value
Patients (*n*)	274	725	
ET (*n*)	274	725	
Total of embryos transferred (*n*)	548	725	
Sac seen at 6 weeks (*n*)	206	363	
Multiple pregnancies at 12 weeks (*n*)	43	4	
Positive pregnancy (*n*)	158	409	
Clinical pregnancy (*n*)	150	363	
On-going pregnancy (*n*)	129	299	

Table 2 Comparison of overall success rates for double cleavage-stage transfer and single blastocyst transfer

	DET day 3	SBT	*p* value
Positive pregnancy/ET rate	57.7%	56.4%	0.722
Clinical pregnancy/ET rate	54.7%	50.1%	0.187
Implantation/embryo transferred rate	37.6%	50.1%	**0.000**
On-going pregnancy/ET rate	47.1%	41.2%	0.096
Multiple pregnancies/clinical pregnancy rate	28.7%	1.1%	**0.000**

ET embryo transfer

Numbers in bold represent statistical significance, $p < 0.05$

Statistical methods

Collected data were analyzed using the SPSS version 26.0. A chi-square test was performed for categorical variables and one-way ANOVA was performed for continuous variables.

Results

Baseline characteristics

Of the 999 women included in the analysis, 274 were DET, and 725 were SBT. The mean ages [SD] of the women were similar in both groups (DET 33.2[2.9] years and SBT 32.9[2.9] years; $p = 0183$). Table 1 shows the baseline characteristics for DET versus SBT. In terms of FET cycles, 400 underwent NC, 243 underwent m-NC, and 356 underwent HRT cycles. Table 2 compares the overall success rates for double cleavage-stage transfer and single blastocyst transfer.

Clinical outcomes

Overall, there were no statistical differences in the rates of positive pregnancy, clinical pregnancy, and ongoing pregnancy between the day 3 DET and SBT groups (Table 1). Implantation rates were significantly higher in

the SBT group (50.1% versus 37.6%, $p < 0.05$). The day 3 DET group had significantly higher multiple pregnancy rates (28.7% versus 1.1%, $p < 0.05$).

Table 3 shows the comparison of patients who underwent NC, m-NC, and HRT cycles either with double embryo transfer on day 3 or single blastocyst transfer.

Subgroup analysis of embryo transfers performed following NC, m-NC, or HRT cycles showed similar results for implantation rates in NC and HRT groups and for multiple pregnancies in all three groups (Table 4). The implantation rates were 34.9% for DET day 3 and 49.7% for SBT ($P = 0.001$) in NC; 40.4% for DET day 3 and 48.6% for SBT ($P = 0.153$) in m-NC; and 38.5% for DET day 3 and 52.0% for SBT ($P = 0.004$) in HRT cycles. The multiple pregnancies rates were 23.2% for DET day 3 and 1.4% for SBT ($P = 0.000$) in NC; 33.3% for DET day 3 and 0.0% for SBT ($P = 0.000$) in m-NC; and 30.9% for DET day 3 and 1.5% for SBT ($P = 0.000$) in HRT cycles.

Discussion

Our real-world study showed that implantation rates during FET were significantly higher with SBT than day 3 DET (50.1% versus 37.6%, $p < 0.05$), which was in line with evidence in the literature which showed SBT to have better implantation rate than day 3 DET [1, 11, 12]. This may be attributed to better-quality embryos if embryo cultures are maintained until day 5 [3, 4].

In our study, the SBT group had significantly lower multiple pregnancy rates than the day 3 DET group (1.1% versus 28.7%, $p < 0.05$). Other studies also demonstrated low multiple pregnancy rate with SBT [3, 13–15]. The patients included in these studies were younger than 36 years while our study included women up to the age of 38 years. Reducing the risk of multiple pregnancies is important due to its medical, psychological, social, and financial implications [14].

Our study did not demonstrate statistical differences in the rates of positive pregnancy, clinical pregnancy, and ongoing pregnancy between SBT and day 3 DET, indicating that positive pregnancy outcomes are possible with SBT [1, 4, 7, 16, 17]. According to Gardner et al. (2000) [17], a good-quality blastocyst can increase the rate of pregnancy by more than 60%. Rao et al. (2021) [1], in a similar study, found similar pregnancy rates for DET and day 5 SBT groups, but the day 6 SBT group had a significantly higher pregnancy rate than either of these groups. Our study did not differentiate the results for day 5 and day 6 groups, as a recent study found comparable pregnancy rates for SBT that were cryopreserved either on day 5 or day 6 [18].

Table 3 Comparison of three endometrial preparation methods between day 3 DET and SBT groups

	Natural cycles		Modified natural cycles		HRT cycles	
	DET day 3 (*n*)	SBT (*n*)	DET day 3 (*n*)	SBT (*n*)	DET day 3 (*n*)	SBT (*n*)
Patients	106	294	68	175	100	256
Embryo transfer	106	294	68	175	100	256
Sacs seen at 6 weeks	74	146	55	85	77	133
Positive pregnancy	57	164	41	92	60	153
Clinical pregnancy	56	146	39	84	55	133
Ongoing pregnancy	52	122	31	62	46	116
Multiple pregnancies at 12 weeks	13	2	13	0	17	2

DET double embryo transfer, *ET* embryo transfer, *HRT* hormone replacement therapy, *SBT* single blastocyst transfer

Table 4 Clinical outcomes after embryo transfer in the day 3 DET and SBT groups

Clinical outcomes	Natural cycles			Modified natural cycles			HRT cycles		
	DET day 3 (%)	SBT (%)	*P* value	DET day 3 (%)	SBT (%)	*P* value	DET day 3 (%)	SBT (%)	*P* value
Positive pregnancy	53.8	55.8	0.721	60.3	52.6	0.278	60.0	59.8	0.968
Clinical pregnancy	52.8	49.7	0.576	57.4	48.0	0.190	55.0	52.0	0.605
Implantation	34.9	49.7	**0.001**	40.4	48.6	0.153	38.5	52.0	**0.004**
Ongoing pregnancy	49.1	41.5	0.178	45.6	35.4	0.144	46.0	45.3	0.907
Multiple pregnancies	23.2	1.4	**0.000**	33.3	0.0	**0.000**	30.9	1.5	**0.000**

DET double embryo transfer, *ET* embryo transfer, *HRT* hormone replacement therapy, *SBT* single blastocyst transfer

Numbers in bold represent statistical significance, $p < 0.05$, a significant difference between DET day 3 and SBT

Higher implantation rates and lower multiple pregnancy rates with SBT were seen in our study regardless of NC, m-NC, and HRT cycles. This may have important implications as patients who underwent HRT cycles are likely to have worse obstetric and perinatal outcomes [19]. Another study also showed no significant difference in live-birth clinical pregnancy rates between NC and m-NC cycles [20].

The key strengths of our study were that the data was current (2019–2021), a large number of patients ($n = 999$) were included, and the inclusion of women up to the age of 38 years. The mean age of women in our study was 33 years, which is higher than the ages of women in similar studies [1, 4]. However, the findings of our study may not be applicable to older women. Another advantage is that as a single-center study, the allocation of patients was done by all physicians following stringent center clinical protocol, and cultures and transfer of embryos were done using the same standard following embryology protocol, thus removing the effects of different culture media and techniques on the study results. This is important as culture media can affect neonatal births [6, 21].

The limitations of our study were its retrospective design and that comparisons between double cleavage transfer and single blastocyst transfer were not done for the same woman. As a retrospective study, patients were allocated to embryo transfer groups according to the physician and the preferences of the patients and were not randomly assigned. However, Stoop et al. (2011) highlighted that physicians and patients may be anxious with a randomized controlled study design [22]. In addition, information on maternal body mass index, smoking status, and alcohol consumption which could affect neonatal outcomes [21], and information on semen quality which could affect blastocyst formation [23] were not collected.

Conclusions

Our retrospective study showed that success rates for both day 3 DET and SBT were comparable. However, SBT is the better choice for embryo transfers than day 3 DET due to SBT's higher implantation rate, comparable pregnancy rate, and significantly lower incidence of multiple pregnancies. These findings are useful for physicians when they counsel patients on expectations of outcomes and make well-informed decisions on the best treatment option available. However, data from prospective, randomized trials and the inclusion of older patients are needed to provide better guidance in clinical practice.

Abbreviations

DET Double embryo transfer
FET Frozen embryo transfer
HRT Hormone replacement therapy
m-NC Modified natural cycle
NC Natural cycle
SBT Single blastocyst transfer

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Authors' contributions

LYH was the main author responsible for the research and writing of the paper. SPK was responsible for data collection and PN was responsible for reviewing and revising the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

No informed consent was required because of the retrospective design of the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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