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Determining Factors Affecting the Successful Outcome of Fresh Embryo Transfer During In Vitro Fertilization: A Retrospective Cohort Study

Chanakarn Suebthawinkul [1*], Pranee Numchaisrika [1], Akarawin Chaengsawang [2], Vijakhana Pilaisangsuree [2], Sadanan Summat [1], Araya Peawdang [1], Konkanok Patchima [1], Punkavee Tuntiviriyapun [1], Paweena Thuwanut [1], Porntip Sirayapiwat [1], Wisan Sereepapong [1]

1- Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
2- King Chulalongkorn Memorial Hospital (KCMH), The Thai Red Cross Society, Bangkok, Thailand

Abstract

Background: Fresh embryo transfer has been decreasing because of advancements in vitrification techniques and safety concerns related to ovarian hyperresponse. However, in normal responders, clinical outcomes appear to be comparable with those with frozen embryo transfer. This study aimed to determine factors that influence successful fresh embryo transfer.

Methods: This retrospective cohort study included 521 women who underwent in vitro fertilization (IVF) and fresh embryo transfer at King Chulalongkorn Memorial Hospital, Thailand. Patients' clinical data, embryo details, endometrial characteristics (thickness and pattern), and embryo transfer procedures (tip and flow during transfer, embryo placement location, difficulty of the procedure, and presence of blood and mucous at catheter) were analyzed. Chi-square test, Fisher's exact test, Student's t-test, and logistic regression were performed for data analysis. A p-value of <0.05 was considered statistically significant.

Results: The overall clinical pregnancy rate was 17.1%. Women aged >40 years were less likely to have a clinical pregnancy than those aged <35 years (adjusted odds ratio [aOR] 0.422; 95% confidence intervals [CI] 0.196-0.908, p=0.027). Day 3 embryo transfer showed a significant decrease in clinical pregnancy compared with blastocyst transfer (aOR 0.514; 95%CI 0.287-0.923, p=0.026). In the subgroup analysis for blastocyst transfer, women with good-quality blastocyst (≥322) were 2.439 times more likely to have a clinical pregnancy than those with poor-quality blastocysts (aOR 2.439; 95%CI 1.199-4.962, p=0.014).

Conclusion: Advanced age and day 3 embryo transfer were significantly associated with low clinical pregnancy rates in fresh embryo transfer.

** Corresponding Author:*
Chanakarn Suebthawinkul, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
E-mail:
chanakarn.su@chula.ac.th

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Introduction

Embryo transfer is an important and final process during in vitro fertilization (IVF) (1, 2). The use of fresh embryo transfer has been decreasing significantly because of improvements in vitrification techniques and safety concerns (3). Despite recent evidence that both fresh or frozen embryo transfer strategies are associated with particular risks, frozen embryo transfer cycle has safety advantages by reducing the chance of ovarian hyperstimulation syndrome, while maintaining a similar or superior success rate in comparison to fresh transfer cycles (3, 4).

Impaired clinical outcomes of fresh embryo transfer during IVF have recently become a concern. It was hypothesized that the supraphysiological hormone levels achieved at the end of ovarian stimulation induce advancement of the endometrium out of the window of implantation. The high level of steroid hormones and the increase in progesterone levels during the later follicular phase may negatively affect endometrial receptivity and embryo implantation (5). However, some evidence shows that fresh transfer is similar to frozen transfer in terms of the ongoing pregnancy rate and cumulative live birth rate in normal responders/overall population (4-6). The cost-effectiveness analysis of the frozen strategy also warrants further clarification. Moreover, fresh embryo transfer provides a shorter time to conception and may be considered an option for patients who have few embryos and have no embryos suitable for freezing (7).

Despite evolutions in IVF technology, including individualized/progestin-prime ovarian stimulation protocol, improved culture system, laboratory techniques, and genetic analysis, embryo transfer, which is the most essential step, appears to receive less attention (8). Even with high-quality embryos, the live birth rate from fresh embryo transfer is relatively low (approximately 20% to 25%) (6, 8). Factors determining successful fresh embryo transfer are still questionable. Although embryo quality mainly influences the implantation outcomes, the success of IVF varies by several factors including the uterine environment and embryo transfer techniques (9). Therefore, this study aimed to determine various factors that affect successful fresh embryo transfer during IVF.

Methods

Study design: This retrospective study was conducted at Infertility Clinic of King Chulalongkorn Memorial Hospital, Thailand. This study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB No. 589/65). Informed consent was waived because of the retrospective nature of the study and the anonymous use of clinical data.

Study population: The medical records of all patients who underwent IVF between January 2013 and October 2022 were reviewed. The inclusion criteria of this study comprised of women aged 20 to 43 years who underwent IVF and fresh embryo transfer. Patients were excluded if they met any of the following criteria: a) body mass index (BMI) >40 kg/m^2 or >35 kg/m^2 with comorbidities, such as hypertension, diabetes, or cardiovascular diseases; b) untreated polyps or myomas distorting the uterine cavity or measuring >5 *cm*; c) intrauterine adhesions; d) recurrent implantation failure; e) congenital uterine malformations; f) undergoing sperm retrieval procedures; or g) more than 2 embryos transferred. The study was limited to women with a fresh embryo transfer over a 10-year period, leaving 521 patients eligible for enrollment in this study. The patients' clinical data (age, BMI, underlying diseases, previous childbirth, cumulative IVF cycle, and presence of myoma or endometrioma), sperm quality (percentage of normal forms during IVF), embryo details (number and stage of transferred embryos), endometrial characteristics (thickness and pattern), and embryo transfer procedures (tip and flow during transfer, embryo placement location, difficulty of the procedure, and presence of blood and mucous in the catheter) were analyzed in this study.

Ovarian stimulation protocols: In this study, ovarian stimulation was performed as previously described (9, 10). The patients were prescribed either recombinant follicle-stimulating hormone (rFSH) or human menopausal gonadotropin (hMG) for ovarian stimulation. The flexible GnRH antagonist protocol was implemented for pituitary suppression. After individualized ovarian stimulation, either recombinant or urinary human chorionic gonadotropin was administered when at least three follicles reached a mean diameter of 18 *mm* on transvaginal sonography (TVS). Oocytes were retrieved 36-37 *hr* after ovulation triggering.

All oocytes were fertilized by intracytoplasmic sperm injection (ICSI) technique. All normally fertilized embryos were cultured in a humidified incubator of 5% CO_2 and 5% O_2 for up to 3-5 days depending on the quality of the embryo. Embryos were graded according to Istanbul Consensus Scoring System (11). The Istanbul System for blastocyst scoring indicates that a score above 322 reflects a good-quality blastocyst. Embryos that failed to reach the cleavage stage, underwent arrest, or experienced apoptosis were discarded prior to embryo transfer. Fresh embryo transfers were performed at the cleavage (days 3-4) or blastocyst (day 5) stage, depending on the number of embryos available for each patient.

Luteal phase support and fresh embryo transfer: All patients were prescribed vaginal progesterone

(Utrogestan; Besins Healthcare, UK) 400 *mg* as an intravaginal suppository, to be taken twice daily starting on the evening of the oocyte retrieval date. Endometrial thickness was evaluated by TVS on the day of ovulation triggering. Four to six days following progesterone exposure, one or two embryos were transferred for each patient under the guidance of transabdominal ultrasonography. In cases where there was difficulty inserting the external catheter into the uterine cavity, a rigid embryo transfer catheter and/or a tenaculum were utilized. The placement location of the embryo in the fundus was evaluated by measuring the distance between the fundal myometrium and endometrial interface and checking for air bubbles, as previously described (9). Pregnancy was confirmed by serial serum β-hCG measurements taken 10-14 days following embryo transfer. Pregnancy outcomes were evaluated including clinical pregnancy measured by fetal heart rate by TVS at around 6-10 weeks, miscarriage rate, as well as the number of live births categorized as term and preterm.

Data collection and statistical analysis: Data were collected and managed using Research Electronic Data Capture (REDCap), a secure web-based software platform hosted at Chulalongkorn University. Data analysis was performed using SPSS software version 22.0 (IBM Corp., USA) and GraphPad Prism version 9.0.1. For descriptive statistics, the mean and standard deviation were used for continuous variables and number and percentage for categorical variables. The normal distribution of data was evaluated using the Shapiro–Wilk test. The patients were divided into two groups for the outcome analysis according to the clinical pregnancy as determined by the fetal heart rate by TVS at about 6-10 weeks. The Chi-square test or Fisher's exact test was used for the comparison of categorical variables, whereas Student's t-test was used for the comparison of continuous variables between the pregnant and nonpregnant groups. Multiple logistic regression analysis was performed to assess the relationship between the collected variables and successful fresh embryo transfer, which defines clinical pregnancy. Crude odds ratios (ORs) were analyzed by univariate logistic regression analysis. Adjusted odds ratios (aORs) were analyzed through multiple logistic regression analysis using backward stepwise selection, and a significance level of $p<0.05$ was applied to the final model. Subgroup analysis was performed particularly in fresh blastocyst transfer. A p-value of <0.05 was considered statistically significant.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board at Chulalongkorn University (IRB No. 589/65).

Results

In total, 521 fresh embryo transfer cycles were analyzed. The patients' clinical data are displayed in tables 1 and 2. The overall clinical pregnancy rate in the fresh transfer cycle was 17.1%. The live birth rate was 12.7%, with 73.3% of pregnancies resulting in term births and 26.7% resulting in preterm births. Of these, the singleton and multiple pregnancy rates were 83.3% and 19.1%, respectively. The miscarriage rate was approximately 43%. The overall clinical pregnancy rates classified by age group were 28.4%, 17.3%, and 11.2% for ages <35, 35-40, and >40 years, respectively. The clinical pregnancy rate reached a peak at 23.7% for day 5 blastocyst transfers, followed by 16.2% for day 4 embryo transfers, and 11.2% for day 3 embryo transfers.

The patients' clinical data, embryo details, and procedural factors between the pregnant and nonpregnant cycles are shown in tables 1 and 2. No significant differences in BMI, previous childbirth, underlying diseases, presence of myoma or endometrioma, cumulative IVF cycle, percentage of normal sperm morphology, number of transferred embryos, endometrial characteristics, endometrial thickness, and procedural factors were found between the two groups. The visual tip of the catheter and injection flow during embryo transfer were observed in all patients in the pregnancy group.

Table 3 demonstrates the association between demographic factors, embryo details, and endometrial and procedural factors and successful outcomes. Women aged 35-40 and over 40 years were significantly associated with lower clinical pregnancy rates compared to those under 35 years. BMI did not reveal a significant association with pregnancy outcomes. Having a previous baby or any underlying diseases was not associated with clinical pregnancy. The presence of myomas greater than 5 *cm* that do not distort the cavity and endometriomas was not significantly associated with clinical pregnancy.

In terms of embryo factors, day 3 embryo trans-

Table 1. Baseline characteristics of participants (n=521)

Characteristics	Pregnancy n (%)	Nonpregnancy n (%)	p-value
n (%)	89 (17.1%)	432 (82.9%)	
Age Mean±SD (years)	37.61±3.93	38.99±3.64	0.002
Age range (years)			
<35	19 (28.4%)	48 (71.6%)	
35-40	50 (17.3%)	234 (82.7%)	0.006
>40	20 (11.2%)	150 (88.2%)	
BMI (kg/m^2)	22.80±3.98	22.08±3.62	0.101
BMI range (kg/m^2) [a]			
Underweight (<18.5)	8 (17.8%)	37 (82.2%)	
Normal (18.5-22.9)	46 (15.2%)	256 (84.8%)	
Overweight (23.0-24.9)	15 (17.6%)	71 (82.4%)	0.602
Obese (≥25.0)	17 (21.5%)	62 (78.5%)	
Missing	3 (33.3%)	6 (66.7%)	
Previous children			
No	87 (17.1%)	411 (82.9%)	
Yes	2 (9.1%)	20 (90.9%)	0.557
Missing	0 (0%)	1 (100%)	
Underlying diseases			
No	80 (16.4%)	400 (83.6%)	
Yes	9 (9.1%)	32 (90.9%)	
Allergic diseases	9 (31.0%)	26 (69.0%)	0.376
GERD	0 (0.0%)	2 (100%)	
Thyroid disease	2 (25.0%)	8 (75%)	
Hyperprolactinemia	0 (0.0%)	2 (100%)	
Presence of non-cavity-distorting myoma [b]			
No	79, (17.8%)	355 (82.2%)	
Yes	10 (11.6%)	76 (88.4%)	0.206
Missing	0 (0%)	1 (100%)	
Presence of endometrioma [b]			
No	75 (17.3%)	358 (82.7%)	
Yes	12 (14.1%)	73 (85.9%)	0.529
Missing	0 (0%)	1 (100%)	
Cumulative IVF cycle			
1st IVF	67 (18.1%)	306 (81.9%)	
2nd IVF	16 (16.8%)	79 (83.2%)	0.142
3rd IVF	3 (6.5%)	43 (93.5%)	
Missing	3 (42.8%)	4 (57.2%)	
Normal morphology of sperm during ICSI (%)	2.85±0.59	2.76±0.61	0.671

Data are presented as mean±SD or n (%). The Chi-square test or Fisher's exact test was used for the comparison of categorical variables whereas Student's t-test was used for the comparison of continuous variables between the pregnant and nonpregnant groups. BMI; body mass index, GERD; gastroesophageal reflux disease, IVF; in vitro fertilization, ICSI; intracytoplasmic sperm injection
a: BMI was classified by World Health Organization Asian-specific BMI (aBMI) classification
b: During the transvaginal ultrasound on the day of ovulation trigger

fer was significantly associated with low clinical pregnancy rates compared with day 5 blastocyst transfer. Similar trends were observed in day 4 embryo transfer; however, it was not significant. Compared with single embryo transfer (SET), double embryo transfer (DET) did not show any significant association with increased clinical pregnancy rates.

Although the average endometrial thickness was similar between the pregnant and nonpregnant groups, cycles with endometrial thickness less than 8 *mm* trended toward low clinical pregnancy rates compared to those with thickness of 8 *mm* or more; however, the difference was not significant. The characteristics of the endometrium, including

Table 2. Embryo characteristics and procedural factors during the embryo transfer

Characteristics	Pregnancy n (%)	Nonpregnancy n (%)	p-value
n (%)	89 (17.1%)	432 (82.9%)	
Stage of embryo			
Day 3	26 (11.2%)	199 (88.8%)	0.003
Day 4	17 (16.2%)	88 (83.8%)	
Day 5	46 (23.7%)	145 (76.3%)	
Number of transferred embryos			
1	24 (15.0%)	136 (85.0%)	0.526
2	65 (17.5%)	296 (82.5%)	
Endometrial thickness (*mm*)	10.34±1.99	10.42±2.57	0.770
Endometrial thickness range			
< 8 *mm*	10 (13.3%)	65 (86.7%)	0.504
≥ 8 *mm*	79 (17.3%)	367 (82.7%)	
Endometrial characteristics			
Triple layer	85 (16.8%)	411 (832%)	0.750
Mix	2 (11.1%)	16 (88.9%)	
Missing	2 (28.5%)	5 (71.5%)	
Tip and flow were seen during embryo transfer			
Yes	84 (16.9%)	409 (83.1%)	0.596
No	0 (0%)	5 (100%)	
Missing	5 (21.7%)	18 (78.3 %)	
Mucous and blood at the inner catheter			
No	77 (16.7%)	385 (83.3%)	0.848
Yes	8 (14.8%)	47 (85.2%)	
Missing	4 (100%)	0 (0%)	
Mucous and blood at the external catheter			
No	52 (20.3%)	204 (79.7%)	0.033
Yes	33 (12.9%)	228 (87.1%)	
Missing	4 (100%)	0 (0%)	
Distance from fundus (*cm*)	1.30±0.37	1.28±0.39	0.795
Distance range (from fundus)			
<1.00 *cm*	13 (14.6%)	76 (85.4%)	0.963
1.00-1.49 *cm*	45 (17.0%)	220 (83.0%)	
1.50-2.00 *cm*	22 (16.4%)	113 (83.6%)	
>2.00 *cm*	2 (15.4%)	13 (84.6%)	
Missing	7 (38.8%)	11 (61.2%)	
Difficulty (change catheter/tenaculum)			
Easy	77 (16.3%)	384 (83.7%)	0.710
Difficult	12 (20.0%)	48 (80%)	

Data are presented as mean±SD or n (%). The Chi-square test or Fisher's exact test was used for the comparison of categorical variables whereas Student's t-test was used for the comparison of continuous variables between the pregnant and nonpregnant groups.

triple and mixed patterns, were not significant predictors of clinical pregnancy.

On the day of embryo transfer, the placement location of the embryo from the fundus was similar between the pregnant and nonpregnant groups. Placement locations were classified into four groups: <1.0, 1.0-1.49, 1.5-2.0, and >2.0 *cm* from the fundus; however, they did not have a significant effect on clinical pregnancy. The presence of mucous and/or blood at the external catheter was associated with low clinical pregnancy rates, whereas the presence of mucous and/or blood at the inner catheter was not associated with pregnancy outcome. The difficulty of the embryo transfer procedure was also not associated with clinical pregnancy.

In this study, the following factors were found to be significant predictors of clinical pregnancy fol-

Table 3. Odds ratio of factors influencing clinical pregnancy of fresh embryo transfer

Factors	Univariable analysis		Multivariable analysis	
	Crude OR (95%CI)	p-value	Adjusted OR [a] (95%CI)	p-value
Age (years)				
<35	1.00	-	1.00	-
35-40	0.529 (0.289-0.993)	0.042	0.563 (0.289-1.009)	0.092
>40	0.320 (0.156-0.655)	0.002	0.422 (0.196-0.908)	0.027
BMI classification				
Underweight (<18.5 kg/m^2)	1.203 (0.495-2.633)	0.661	-	-
Normal (18.5-22.9 kg/m^2)	1.00		-	-
Overweight (23.0-24.9 kg/m^2)	1.193 (0.612-2.219)	0.590	-	-
Obese (≥25.0 kg/m^2)	1.526 (0.803-2.801)	0.183	-	-
Having previous children	0.484 (0.076-1.699)	0.333	-	-
Having underlying disease	1.485 (0.644-3.125)	0.320	-	-
Presence of non-cavity distorting myoma [c]	0.607 (0.284-1.176)	0.164	-	-
Presence of endometrioma [c]	0.785 (0.389-1.469)	0.471	-	-
Stage of embryo				
Day 3	0.405 (0.235-0.685)	<0.001	0.514 (0.287-0.923)	0.026
Day 4	0.623 (0.328-1.137)	0.133	0.741 (0.378-1.452)	0.382
Day 5	1.00	-	1.00	-
Number of transferred embryos				
1	1.00	-	-	-
2	1.206 (0.731-2.044)	0.473	-	-
Endometrial thickness range				
< 8 *mm*	0.733 (0.341-1.432)	0.392	-	-
≥ 8 *mm*	1.00	-	-	-
Endometrial characteristics				
Triple	1.00	-	-	-
Mix	0.619 (0.097-2.231)	0.528	-	-
Distance range (from fundus)				
<1.00 *cm*	0.733 (0.325-1.573)	0.436	-	-
1.00-1.49 *cm*	1.047 (0.604-1.857)	0.873	-	-
1.50-2.00 *cm*	1.00	-	-	-
>2.00 *cm*	0.909 (0.135-3.698)	0.906	-	-
Presence of mucous and blood at the inner catheter	0.870 (0.368-1.822)	0.729	-	-
Presence of mucous and blood at the external catheter	0.592 (0.365-0.948)	0.031	-	-
Difficulty (change catheter/tenaculum)	1.173 (0.557-2.288)	0.655	-	-

a: Variables entered on step 1: Age, BMI, previous children, underlying diseases, presence of myoma and endometrioma, stage of the embryo, number of the transferred embryo, endometrial thickness and characteristic, mucous and blood at inner and external catheter, embryo placement location, and the difficulty of the procedure. Crude OR analyzed by univariable logistic regression analysis. Adjusted OR analyzed by multiple logistic regression analysis using backward stepwise method
b: BMI was classified by World Health Organization Asian-specific BMI (aBMI) classification
c: During the transvaginal ultrasound on the day of ovulation trigger
BMI: Body Mass Index, GERD: Gastroesophageal Reflux Disease, IVF: In Vitro Fertilization, OR: Odds Ratio, CI: Confidence Interval

lowing fresh embryo transfer: age, embryo stage, and presence of mucous and/or blood at the external catheter. Factors that showed clinically or statistically significant associations with pregnancy outcomes were included in the analysis model using backward stepwise method. After adjusting for all factors, age and embryo stage were found to be significant predictors of clinical pregnancy (Table 3).

Women aged >40 years were approximately 58% less likely to have a clinical pregnancy than those aged <35 years. A similar trend was observed in women aged 35-40 years in whom the clinical pregnancy rate decreased by 44% compared with those aged <35 years; however, the difference was not significant. When compared with the normal BMI group, the underweight, overweight, and obese groups did not show any difference in clinical pregnancy rates. Compared with day 5 blastocyst transfer, day 3 embryo transfer showed a significant association with low clinical pregnancy rates by approximately 49%,

Table 4. Odds ratio of factors influencing clinical pregnancy of day 5 fresh blastocyst transfer (n=191)

Factors	Univariable analysis		Multivariable analysis	
	Crude OR (95%CI)	p-value	Adjusted OR [a] (95%CI)	p-value
Age (years)				
<35	1.00	-	-	-
35-40	0.548 (0.245-1.250)	0.145	-	-
>40	0.514 (0.186-1.371)	0.188	-	-
BMI classification				
Underweight (<18.5 *kg/m²*)	1.415 (0.463-3.902)	0.517	-	-
Normal (18.5-22.9 *kg/m²*)	1.00	-	-	-
Overweight (23.0-24.9 *kg/m²*)	1.200 (0.429-3.073)	0.713	-	-
Obese (≥25.0 *kg/m²*)	1.698 (0.657-4.180)	0.258	-	-
Having previous children	0.389 (0.020-2.207)	0.380	-	-
Having underlying diseases	0.527 (0.028-3.198)	0.558	-	-
Presence of non-cavity distorting myoma [c]	0.792 (0.174-2.636)	0.727	-	-
Presence of endometrioma [c]	0.846 (0.231-2.484)	0.776	-	-
Number of transferred embryos				
1	1.00	-	-	-
2	0.875 (0.398-2.047)	0.747	-	-
Quality of blastocyst				
Not good	1.00	-	1.00	-
Good (≥322)	2.439 (1.218-5.085)	0.014 *	2.439 (1.199-4.962)	0.014
Endometrial thickness range				
<8 *mm*	0.668 (0.149-2.170)	0.542	-	-
≥8 *mm*	1.00	-	-	-
Endometrial characteristics				
Triple	1.00	-	-	-
Mix	1.093 (0.053-8.783)	0.939	-	-
Distance range (from fundus)				
<1.00 *cm*	0.375 (0.082-1.264)	0.147	-	-
1.00-1.49 *cm*	1.059 (0.512-2.231)	0.876	-	-
1.50-2.00 *cm*	1.00	-	-	-
>2.00 *cm*	1.000 (0.137-4.862)	0.990	-	-
Presence of mucous and blood at the inner catheter	0.718 (0.199-2.062)	0.569	-	-
Presence of mucous and blood at the external catheter	0.571 (0.272-1.152)	0.125	-	-
Difficulty (change catheter/tenaculum)	1.603 (0.533-4.355)	0.370	-	-

a: Variables entered on step 1: Age, BMI, previous children, underlying disease, presence of myoma and endometrioma, stage of the embryo, number of the transferred embryo, endometrial thickness and characteristic, mucous and blood at inner and external catheter, embryo placement location, and the difficulty of the procedure. Crude OR analyzed by univariable logistic regression analysis. Adjusted OR analyzed by multiple logistic regression analysis using backward stepwise method
b: BMI was classified by World Health Organization Asian-specific BMI (aBMI) classification
c: During the transvaginal ultrasound on the day of ovulation trigger
BMI: Body Mass Index, GERD: Gastroesophageal Reflux Disease, IVF: In Vitro Fertilization, OR: Odds Ratio, CI: Confidence Interval

whereas the association noted for day 4 embryo transfer was not different from that of day 5 blastocyst transfer. Cycles with DET did not show any difference in clinical pregnancy compared with cycles with SET. However, the multiple pregnancy rate was significantly higher in DET than in SET (21.9% *vs*. 0%). After adjusting for all the above factors, endometrial thickness/pattern and presence of mucous and/or blood at the external catheter were not significantly associated with pregnancy outcomes.

Subgroup analysis of fresh blastocyst transfer: Table 4 presents the association between the related factors and successful outcomes after day 5 fresh blastocyst transfer. After adjusting for clinically and statistically relevant factors, the quality of the transferred blastocysts was determined to be significantly associated with pregnancy outcomes. Only blastocyst quality presented a significant association with clinical pregnancy. Good-quality blastocysts (Istanbul scoring ≥322) revealed a 2.439 times higher chance of pregnancy than poor-quality blastocysts.

Discussion

In this study, the clinical pregnancy rate in fresh embryo transfer cycles was 17.1%. Advanced age and day 3 embryo transfer were significantly as-

sociated with low clinical pregnancy rates. The clinical pregnancy rates of IVF and fresh embryo transfer cycles were lower and miscarriage rates were higher in this study than reported in previous studies (12-15). This is likely due to the fact that majority of our study participants (87%) were older than those in previous studies, with most being over 35 years of age. Additionally, the transferred embryos were not examined for chromosomal analysis. However, the age-group-specific clinical pregnancy rate was comparable to that reported in earlier research (9, 16). Our finding confirmed that age is an important predictor of pregnancy outcomes. Reproductive aging is associated with decreased oocyte quality, increased aneuploidy, and decreased fertility in animals and humans because of several factors, such as weakened chromosome cohesion, recombination defects, and age-associated spindle dysfunction during oocyte meiosis (17). Similar to our previous study, female aging is a strong predictor of inferior outcomes for frozen embryo transfer (9). The rates of IVF success and miscarriage are also dependent on age, and maternal and fetal adverse outcomes dramatically increase with advanced age (16, 18).

Compared with our data in the frozen cycle, fresh embryo transfer also demonstrated a lower overall clinical pregnancy rate (32.4% *vs*. 17.1%) and a higher miscarriage rate (31.8% *vs*. 43.8%) (9). The inferiority of pregnancy outcomes in fresh cycles is biologically attributed to the supraphysiological level of steroid hormones during ovarian stimulation, which impairs endometrial receptivity and negatively affects implantation (5, 6). Furthermore, one possible explanation is that physicians tend to perform fresh embryo transfers in patients with poor prognoses or in cases where there are no suitable embryos available for cryopreservation. Although the pregnancy rate of frozen transfer appears superior to that of fresh transfer in our center, concerns remain about the obstetric and perinatal outcomes, such as hypertensive disorders of pregnancy and large-for-gestational-age newborns. Additionally, further investigation is needed to determine whether frozen transfers can enhance cost-effectiveness and benefit poor responders (5, 19).

In this study, cleavage embryo (day 3) transfer was significantly associated with lower pregnancy rates compared to blastocyst transfer. This finding supports the results of a recent meta-analysis involving 1784 fresh cycle transfers that showed a superior clinical pregnancy rate of approximately 1.26 times in the blastocyst stage compared with cleavage stage transfer (20). Glujovsky et al. showed the superiority of blastocyst transfer to cleavage transfer in improving the live birth rates in the fresh cycles at approximately 27% (21). This result is most likely due to the evidence that blastocyst transfer provides better physiological synchronization with the endometrial receptivity of the uterus and prevents the embryos from exposure to high levels of estrogen and progesterone during ovarian stimulation (22). Additionally, blastocyst transfer allows embryos with the greatest potential for continued development to be naturally selected for transfer (8, 23). In comparison to cleavage embryos, it also indicates a decreased likelihood of aneuploidy in day 5 embryos. However, a higher cancellation rate due to the lack of available embryos and a lower cryopreservation rate were noted in blastocyst transfer (20).

Morphological grading of the blastocyst is another important predictor of successful outcomes. Good-quality blastocysts (Istanbul scoring ≥322) were associated with a 2.44 times higher chance of pregnancy than poor-quality blastocysts in fresh transfer (Table 4, p=0.014). Similar to our previous study, the morphological grading of blastocysts after thawing contributed to the positive association with successful implantation (9). This finding supports the results of these retrospective analyses of the characteristics of blastocysts from over 4,000 single blastocyst transfers, which indicate that the stage of blastocyst expansion and trophectoderm grading are highly significant, independent predictors of clinical pregnancy and live birth in fresh cycles (24, 25). Another cohort study analyzing 5,653 blastocysts found that blastocyst scores derived from morphological components were associated with successful implantation and euploid prediction (26). Morphological evaluation of blastocysts remains the gold standard for prioritization and selection of the best embryo to transfer, particularly in the fresh cycle, in which preimplantation genetic testing may not be performed.

After adjusting for other confounders, BMI, number of transferred embryos, and endometrial thickness did not demonstrate a significant association with pregnancy outcomes. Several studies have suggested that female obesity detrimentally affects live birth rates and increases miscarriage rates following IVF by several mechanisms, such as the adverse effects of obesity on ovarian func-

tion, embryo quality, and endometrium receptivity (27-29). However, some studies have shown that in fresh transfer, patients who are obese have IVF outcomes comparable with those with normal BMI, except for those with BMI >40 kg/m^2, which was further supported by our study (30, 31).

Regarding the number of transferred embryos, our findings support a recent meta-analysis that showed similar pregnancy, live birth, and cumulative live birth rates between DET and SET (32, 33). However, SET shows a safety advantage by lowering the risk of multiple pregnancies and optimizing the perinatal outcomes of the mother and baby (34). Although numerous studies have investigated the relationship between endometrial thickness and IVF outcomes, no consensus has been reached. In a previous study of 756 patients in their first fresh IVF/ICSI, a significant decrease in implantation and clinical pregnancy rates was noted in patients with endometrial thickness of <8 *mm* compared with those with ≥8 *mm* (14). Another study showed that clinical pregnancy and live birth rates decreased with each millimeter decline in endometrial thickness (<8 *mm*) in the fresh cycle (35). In contrast, a large cohort study evaluating 42,132 fresh cycles found that endometrial thickness demonstrated a curvilinear relationship with pregnancy outcomes, and the ideal range of endometrial thickness in fresh cycles was approximately 12-15 *mm* (15). Nevertheless, no association of either endometrial thickness or pattern with pregnancy outcomes was observed in the current study. This is probably because the cutoff point to define a thin endometrium is controversial, and very few cycles had an endometrial thickness of <8 *mm* in this study.

The strength of this study is the amalgamation of all relevant factors and the analysis of a total of 521 fresh cycles. Although this study has a retrospective design, the incomplete data was <5% in our study. The effects of confounders were reduced by adjusting for all possible confounding factors. Despite earlier research on this subject, our study is the first to assess the factors that influence the successful transfer of fresh embryos in Thailand and Southeast Asia. In addition, subgroup analysis was conducted for fresh blastocyst transfer and standard measurement to identify the location of the transferred embryo. Finally, compared to previous studies in the younger age groups, the majority of the participants in our study were over 35, which is a good representation of the infertile population in the contemporary context.

The limitation of this study was the analysis of information collected based on a single tertiary university hospital and the non-randomized design, which may have introduced some biases. The ovarian stimulation parameters, such as the dosage, types of exogenous gonadotropins, the duration of stimulation, and other semen parameters including concentration, motility, and progressive motility were not included in this analysis. Lastly, the observations in our study were based on fresh cycles of IVF/ICSI; whether this holds true for frozen embryo transfer warrants further investigation. Despite the retrospective design, this study still provides valuable information for clinicians and patients regarding the factors that influence the pregnancy outcomes following fresh embryo transfer.

Conclusion

Low clinical pregnancy rates in fresh embryo transfer procedures were significantly correlated with advanced age (>40 years) and day 3 embryo transfer. Compared to women <35, those > 40 had about 58% lower chance of becoming pregnant. Day 3 embryo transfer was significantly associated with lower clinical pregnancy rates by roughly 49% as compared to day 5 blastocyst transfer. A better understanding of factors that influence successful embryo transfer in fresh cycles is critical to guiding clinical practice and effectively counseling patients, which can ultimately improve the outcomes of infertility treatment.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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