

Original Research

Single Blastocyst-Stage Embryo Transfer should be Promoted for IVF Cycles Using Donor Sperm (IVFD)

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Abstract

Background: Twin pregnancies carry significant fetal, perinatal and maternal risks. Thus, it is important to evaluate clinical outcomes of *in vitro* fertilization with donor sperm (IVFD) in the first attempt with double cleavage-stage embryos and explore optimal number of day 3 high-quality embryos for the blastocyst transfer on day 5. **Methods:** We retrospectively identified all IVFD embryo transfers (IVFD-ETs) for the initial time between 2014 and 2021 at our hospital. We mainly analyzed the twin pregnancy rates for double day 3 embryo transfers and clinical outcomes of day 5 blastocyst transfers by prolonged culture with different numbers of day 3 high-quality embryos. **Results:** Among 1512 IVFD-ETs, 834 were day 3 embryo transfers and 678 were day 5 blastocyst transfers. Our data indicated that the twin pregnancy rates reached up to 40% in the 674 double cleavage-stage embryo transfers and it was not due to the quality of the transferred embryos. For prolonged culture with 2, 3 and 4 day 3 high-quality embryos, the cycle rates of no high-quality blastocysts obtained were 30.43%, 19.80% and 7.91%, respectively. The clinical pregnancy rates were 56.52%, 74.26% and 72.32%, respectively. The twin pregnancies rates were 7.69%, 14.67% and 6.77%, respectively. **Conclusions:** Transferring double cleavage-stage embryos had high risks of twin pregnancies in the IVFD-ETs. Blastocyst transfer was safe and recommended for the patients with three or more high-quality embryos on day 3.

Keywords: IVF; donor sperm; blastocyst transfer; twin pregnancy

1. Introduction

Infertility affects approximately 15% of couples. The requirement of *in vitro* fertilization (IVF) treatments using donor semen has gradually increased in recent years. In China, treatments with donor sperm were mainly due to azoospermia and intracytoplasmic sperm injection (ICSI) failures.

It was increasingly believed that the singleton pregnancy was the only desirable outcome of assisted reproductive technology. Nevertheless, the twin pregnancy rates increased by 50–70% in the last three decades [1]. Twin pregnancies carry significant fetal, perinatal and maternal risks. The incidence of virtually each obstetrical complication was more frequent in multiple pregnancies. It has been suggested that the norm be 1 embryo transferred at a time [2].

Embryo quality is a significant factor for achieving a live birth by assisted reproductive technology (ART). However, previous research has mainly focused on the effect of oocyte quality on embryo quality. Recently, the onus for improving embryo quality was also equally shared by the quality of sperm [3]. In women undergoing IVF, the use of donor sperm improved pregnancy outcomes as compared to those using partner sperm. One reason was that donor sperm had better sperm quality compared to partner sperm. Alternatively, the proportion of women who had fertility problems was lower in IVF with donor sperm (IVFD) com-

pared with conventional IVF cycles. There was a high risk of twin pregnancies if double embryos were transferred in the IVFD embryo transfers (IVFD-ETs). Therefore, the aim of this study was to explore the clinical outcomes of IVFD-ETs with double cleavage-stage embryos and identify optimal number of day 3 high-quality embryos for prolonged culture to the blastocyst-stage.

2. Materials and Methods

2.1 Study Design and Participants

This study included 1512 IVFD-ETs for the initial transfer from January 2014 to January 2021 at Northwest Women's and Children's Hospital. Eight hundred thirty-four were cleavage-stage embryo transfer which included six hundred seventy-four double embryo transfers. Double embryo transfers were assigned into the D3-DET-HH group (transfer of day 3 two high-quality embryos; $n = 200$), D3-DET-HL (transfer of day 3 one high-quality and one low-quality embryo; $n = 98$), and D3-DET-LL (transfer of day 3 two low-quality embryos; $n = 138$) groups. Six hundred seventy-eight were blastocyst transfers which included 76 embryo transfers with no high-quality blastocysts obtained. The blastocyst transfers were assigned into the D5-ET-2H group (transfers by extended culture to blastocyst-stage with day 3 two high-quality embryos; $n = 46$), D5-ET-3H (transfers by extended culture to blastocyst-stage with day 3 three high-quality embryos; $n = 101$) and D5-ET-



≥4H groups (transfers by extended culture to blastocyst-stage with day 3 more than three high-quality embryos; n = 531) groups. The inclusion criteria were as follows: (1) women aged ≤38 years old; (2) first ovarian stimulation cycle; (3) fresh embryo transfer. Exclusion criteria were as follows: (1) endometriosis; (2) intrauterine adhesions; (3) polycystic ovary syndrome (PCOS); (4) *in vitro* maturation (IVM) cycle; (5) rescue ICSI cycle with donor sperm; (6) no embryo available on day 3. This research was approved by the ethics committee of Northwest Women's and Children's Hospital (No. 2022007).

2.2 Stimulation Protocol

The controlled ovarian hyperstimulation (COH) was described previously [4]. Notably, recombinant follicle-stimulating hormone (FSH) or urinary FSH and/or human menopausal gonadotropins were used with daily doses between 100 and 450 IU based on patients' characteristics as calculated previously [4].

2.3 Sperm Preparation and IVFD Procedure

Donor's semen samples were obtained from Shaanxi Province Human Sperm Bank. The general procedures for donor recruitment, sperm freezing, and selection of recipients were in accordance with the standards of National Health and Family Planning Commission (NHFPC) of the People's Republic of China. The semen parameters for donors before freezing are: volume ≥2.0 mL, concentration ≥60 million/mL, progressive motility ≥60%, normal sperm morphology ≥70%; and after thawing are: concentration ≥15 million/mL, progressive motility ≥32%, normal sperm morphology ≥4% [5]. Conventional IVF was performed 39–41 h after human chorionic gonadotropin (hCG) injection and each oocyte was incubated with a concentration of 50,000 motile sperm/mL.

2.4 Embryo Assessment

After 64 to 68 h of culture, the cleavage-stage embryos were scored according to homogeneous degree of blastomeres, the number of blastomeres and embryo fragmentation. The high-quality cleavage-stage embryos were graded I and II. The available cleavage-stage embryos were graded I, II, and III [6]. The blastocyst-stage embryos were scored according to the stage of development from 1 to 6, the grade of the inner cell mass and the trophectoderm [7]. The high-quality blastocyst was graded ≥3BB.

2.5 Embryo Transfer (ET) and Pregnancy Confirmation

Patients was given 60 mg progesterone (Xianju Pharmaceutical Co., Ltd., Taizhou, Zhejiang, China) by intramuscular injection or 600 mg progesterone of vaginal micronized daily post embryo transfer. An ET catheter (Cook, Limerick, Ireland) was utilized to place the embryos with the guidance of transabdominal ultrasound. To increase the success rate of embryo placement, the mucus in the cervi-

cal os was cleaned in advance with a cotton swab soaked in warm and humid saline. Luteal support was provided for all patients after ET. Clinical pregnancy was characterized as the presence of an intrauterine gestational sac on ultrasonography during the first trimester. Ongoing pregnancy was defined as a clinical pregnancy that continued for at least 12 weeks.

2.6 Statistical Analysis

Statistical analysis between groups in the case of continuous variables was performed with Student's *t* test for data with normal distribution. Non-parametric Mann-Whitney U-test was performed for data with skewed distribution. Statistical analysis between groups in the case of categorical variables was expressed as number and percentage and Chi-square test or Fisher exact test was performed. Forward logistic regression analysis was performed to determine the risk factors for twin pregnancy. The statistical analysis was performed with SPSS version 23 (IBM Corp., Armonk, NY, USA). $p < 0.05$ were considered statistically significant.

3. Results

3.1 Study Design

The study flow chart is shown in Fig. 1. After excluding subjects with various factors, 1512 women underwent IVFD-ETs for the initial time during the study period. Eight hundred thirty-four were cleavage-stage embryo transfers which included 674 double embryo transfers. Six hundred seventy-eight were blastocyst-stage embryo transfers which included 76 embryo transfers with no high-quality blastocysts obtained.

3.2 Baseline Characteristics and Clinical Outcomes of Single Day 3 Embryo Transfers

Our data showed no significant differences in the aspects of female age, body mass index (BMI), basal FSH value and endometrial thickness between D3-SET-H (transfer of day 3 single high-quality embryos) and D3-SET-L (transfer of day 3 single low-quality embryos groups) ($p > 0.05$). The number of retrieved oocytes was significantly higher in the D3-SET-H group than that in the D3-SET-L group (7.00 ± 4.03 versus 5.57 ± 3.2 ; $p < 0.05$). The clinical pregnancy (53.85% versus 28.57%; $p < 0.05$), ongoing pregnancy (50.00% versus 28.57%; $p < 0.05$) and live birth (49.04% versus 26.79%; $p < 0.05$) rates were significantly higher after transferring single day 3 high-quality embryos than transferring single day 3 low-quality embryos (Table 1).

3.3 Baseline Characteristics and Clinical Outcomes of Double Day 3 Embryo Transfers

The twin pregnancy rates at the initial transfer with double cleavage-stage embryos reached 41.38% in the IVFD-ETs (Fig. 2). The number of retrieved oocytes was

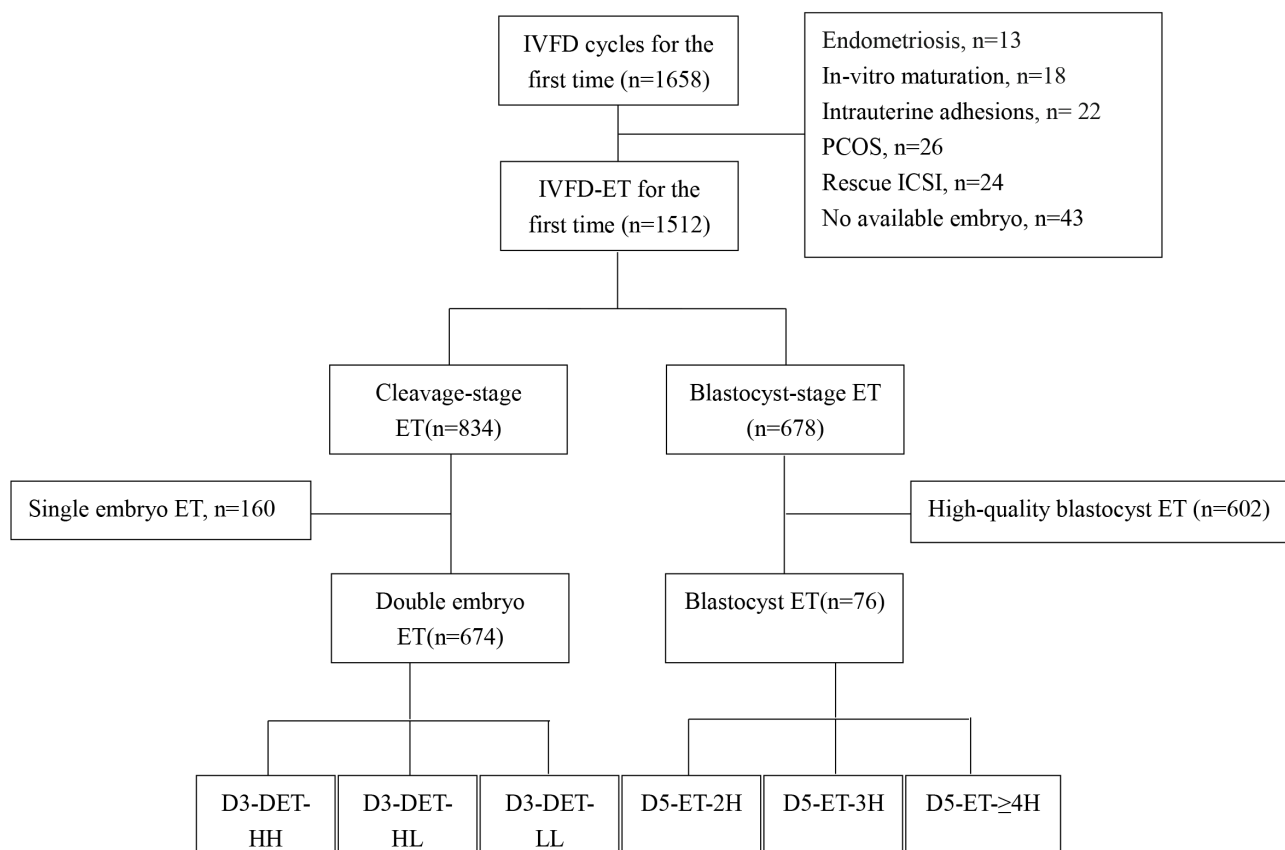


Fig. 1. Study flow chart. IVFD, *in vitro* fertilization with donor sperm; ET, embryo transfer; PCOS, polycystic ovary syndrome; ICSI, intracytoplasmic sperm injection; D3-DET-HH, transfer of day 3 two high-quality embryos; D3-DET-HL, transfer of day 3 one high-quality and one low-quality embryo; D3-DET-LL, transfer of day 3 two low-quality embryos; D5-ET-2H, transfers by extended culture to blastocyst-stage with day 3 two high-quality embryos; D5-ET-3H, transfers by extended culture to blastocyst-stage with day 3 three high-quality embryos; D5-ET- \geq 4H, transfers by extended culture to blastocyst-stage with day 3 more than 3 high-quality embryos.

significantly higher in the D3-DET-HH group than that in the D3-DET-HL and D3-DET-LL groups (9.99 ± 3.90 versus 8.38 ± 4.29 and 8.64 ± 4.07 ; $p < 0.05$). The clinical pregnancy rate of the D3-DET-HH, D3-DET-HL and D3-DET-LL groups was 75.00%, 64.77% and 50.51% which showed significant differences among the three groups ($p < 0.05$). The D3-DET-HH and D3-DET-HL groups showed significantly higher ongoing pregnancy (68.75% and 62.50% versus 46.46%; $p < 0.05$) and live birth (65.50% and 59.66% versus 45.45%; $p < 0.05$) rates than D3-DET-LL group. The twin pregnancy rates were comparable among the three groups (40.67% versus 41.23% versus 46.00%; $p > 0.05$) (Table 2). Potential factors were entered as independent variables in the multivariate logistic regression analysis for twin pregnancies and we observed that no risk factor was independently associated with twin pregnancies (Table 3).

3.4 Baseline Characteristics and Clinical Outcomes of Blastocyst-Stage Embryo Transfers

The patients in the D5-ET-2H group were significantly older (29.89 ± 3.94 versus 28.78 ± 3.57 ; $p < 0.05$) and had

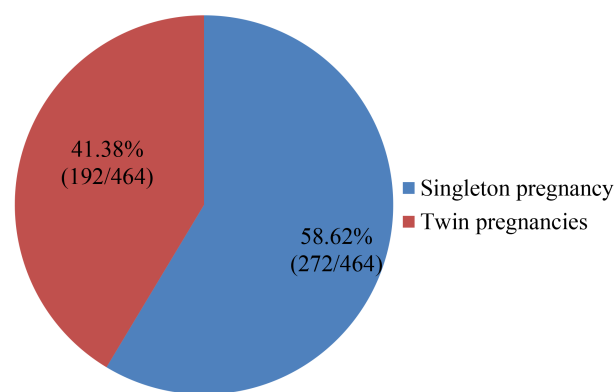


Fig. 2. Proportion of twin pregnancies at initial embryo transfer with double cleavage-stage embryos in the IVFD cycles.

higher basal FSH value (7.77 ± 3.78 versus 6.84 ± 2.58 ; $p < 0.05$) than those in D5-ET- \geq 4H group. The mean number of retrieved oocytes of D5-ET-2H, D5-ET-3H and D5-ET- \geq 4H groups was 7.83, 9.23 and 12.15, which demonstrated significant differences among the three groups ($p < 0.05$).

Table 1. Baseline characteristics and clinical outcomes of the study population.

Parameter	D3-SET-H	D3-SET-L	<i>p</i>
No. transfers	104	56	/
Female age (years)	30.16 ± 4.29	29.32 ± 4.24	0.237
BMI (kg/m ²)	22.72 ± 3.73	22.44 ± 3.19	0.811
Basal FSH (mIU/mL)	7.45 ± 2.39	8.20 ± 3.56	0.385
No. of retrieved oocytes (n)	7.00 ± 4.03*	5.57 ± 3.22*	0.024
Endometrial thickness (mm)	11.89 ± 2.24	11.84 ± 2.64	0.862
Clinical pregnancy (% , n)	53.85% (56/104)*	28.57% (16/56)*	0.002
Ongoing pregnancy (% , n)	50.00% (52/104)*	28.57% (16/56)*	0.009
Live birth (% , n)	49.04% (51/104)*	26.79% (15/56)*	0.006

D3-SET-H, transfer of day 3 single high-quality embryos; D3-SET-L, transfer of day 3 single low-quality embryos; BMI, body mass index; FSH, follicle-stimulating hormone; *significantly different.

Table 2. Baseline characteristics and clinical outcomes of the study population.

Parameter	D3-DET-HH	D3-DET-HL	D3-DET-LL	<i>p</i>
Number of transfers	400	176	98	/
Maternal age (years)	28.94 ± 4.00	29.24 ± 4.49	29.14 ± 4.65	0.674
BMI (kg/m ²)	21.92 ± 2.99	21.64 ± 2.70	22.03 ± 3.15	0.231
Total Gn dosage (IU)	2214.94 ± 822.16 ^a	2439.06 ± 945.01 ^a	2381.38 ± 869.67	0.004
Stimulation duration (days)	10.35 ± 2.17	10.25 ± 2.06	10.35 ± 1.83	0.617
FSH (mIU/mL)	6.97 ± 2.23	7.37 ± 2.83	7.44 ± 2.94	0.070
Number of oocytes retrieved (n)	9.99 ± 3.90 ^{a,b}	8.38 ± 4.29 ^a	8.64 ± 4.07 ^b	<0.001
Endometrial thickness (mm)	11.80 ± 2.78	11.89 ± 2.22	11.79 ± 2.02	0.626
Clinical pregnancy (% , n)	75.00 (300/400) ^{a,b}	64.77 (114/176) ^{a,c}	50.51 (50/99) ^{b,c}	<0.001
Twin pregnancies (% , n)	40.67 (122/300)	41.23 (47/114)	46.00 (23/50)	0.479
Ongoing pregnancy (% , n)	68.75 (275/400) ^a	62.50 (110/176) ^b	46.46 (46/99) ^{a,b}	<0.001
Live birth (% , n)	65.50 (262/400) ^a	59.66 (105/176) ^b	45.45 (45/99) ^{a,b}	<0.001

^a versus ^a, ^b versus ^b, ^c versus ^c were significantly different. Gn, gonadotropin.

Table 3. Multivariate logistic regression analysis of risk factors for twin pregnancies.

	OR	95% CI	<i>p</i>
Age (years)	0.981	0.933–1.031	0.450
BMI (kg/m ²)	1.026	0.960–1.096	0.447
Total Gn dosage (IU)	1.000	1.000–1.000	0.770
Gn stimulation (days)	1.024	0.912–1.150	0.688
Basal FSH (mIU/mL)	1.020	0.938–1.109	0.641
No. of retrieved oocytes (n)	0.979	0.930–1.030	0.415

OR, odds ratio; 95% CI, 95% confidence interval.

The patients in the D5-ET-≥4H group showed significantly lower cycle rate of inadequate blastocyst than that in the D5-ET-2H and D5-ET-3H groups (7.91% versus 30.43% and 19.80%; *p* < 0.05). The patients in the D5-ET-2H group showed a significantly lower clinical pregnancy rate than that in the D5-ET-3H and D5-ET-≥4H groups (56.52% versus 74.26% and 72.32%; *p* < 0.05). The patients in the D5-ET-3H group showed significantly higher twin pregnancy rates than that in D5-ET-≥4H group (14.67% versus 6.67%; *p* < 0.05). No significant difference was observed in the ongoing pregnancy rate among the three groups (54.35%

versus 65.35% and 65.91%; *p* > 0.05). The patients in the D5-ET-2H group showed significantly lower live birth rates than those in the D5-ET-≥4H group (47.83% and 63.09%; *p* < 0.05) (Table 4). For patients with two high-quality embryos on day 3, the D3-DET-HH group showed significantly higher clinical pregnancy (75.00% versus 56.52%; *p* < 0.05), twin pregnancies (40.67% versus 7.69%; *p* < 0.001), ongoing pregnancy (68.75% versus 54.35%; *p* < 0.05) and live birth (65.50% versus 47.83%; *p* < 0.05) rates than the D5-ET-2H group (Fig. 3).

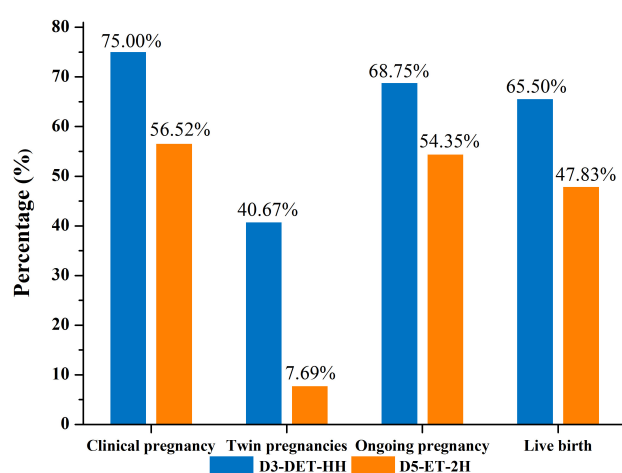
4. Discussion

In this research, our data revealed that the clinical pregnancy rate was only 28.57% for single low-quality cleavage-stage embryo transfer. Double embryo transfers have been used to increase the live birth rate in conventional IVF strategy. Thus, double cleavage-stage embryo transfers were previously recommended to ensure ideal success rate on day 3 in our center. The increased number of transferred embryos was associated with a higher incidence of multiple births. Multiple pregnancies increased the incidence of low birth weight, perinatal mortality and congenital malformations [8].

Table 4. Baseline characteristics and clinical outcomes of the study population.

Parameter	D5-ET-2H	D5-ET-3H	D5-ET-≥4H	p
No. transfers	46	101	531	/
Female age (years)	29.89 ± 3.94 ^a	29.54 ± 3.43	28.78 ± 3.57 ^a	0.047
BMI (kg/m ²)	24.73 ± 3.74	22.18 ± 2.91	22.38 ± 3.15	0.710
Basal FSH (mIU/mL)	7.77 ± 3.78 ^a	7.33 ± 2.07	6.84 ± 2.58 ^a	0.027
No. of retrieved oocytes (n)	7.83 ± 4.00 ^{a,b}	9.23 ± 3.72 ^{a,c}	12.15 ± 3.70 ^{b,c}	<0.001
Endometrial thickness (mm)	12.10 ± 2.72	11.87 ± 2.56	12.05 ± 2.50	0.917
No high-quality blastocyst obtained (n, %)	30.43 (14/46) ^a	19.80 (20/101) ^b	7.91 (42/531) ^{a,b}	<0.001
Clinical pregnancy (%), n)	56.52 (26/46) ^{a,b}	74.26 (75/101) ^a	72.32 (384/531) ^b	0.023
Twin pregnancy (%), n)	7.69 (2/26)	14.67 (11/75) ^a	6.77 (26/384) ^a	0.022
Ongoing pregnancy (%), n)	54.35 (25/46)	65.35 (66/101)	65.91 (350/531)	0.115
Live birth (%), n)	47.83 (22/46) ^a	60.40 (61/101)	63.09 (335/531) ^a	0.041

^a versus ^a, ^b versus ^b, ^c versus ^c were significantly different.

**Fig. 3. Clinical outcomes of the study population.**

It has been reported that pregnancies conceived with donor sperm had a lower odds of early pregnancy loss and ectopic pregnancy compared to pregnancies conceived with partner sperm [9]. Bortoletto *et al.* [10] indicated that the women using donor sperm were more likely to have a live birth than those using their partner's sperm. Thus, multiple births are more likely to occur in the IVFD-ETs than conventional IVF-ETs when double embryos were transferred. A recent meta-analysis indicated that single blastocyst transfer was associated with higher clinical pregnancy and live birth rates compared to single cleavage-stage embryo transfer [11]. Therefore, it is important to evaluate clinical outcomes of double cleavage-stage embryo transfers and identify optimal number of high-quality cleavage-stage embryos for extended culture to blastocyst-stage in the IVFD-ETs.

Our data indicated that clinical pregnancy and live birth rates were significantly improved by adding an extra high or low-quality embryo on day 3. However, the risks of multiple pregnancies were also increased. Previous research also showed that the transfer of an extra poor quality embryo might increase the incidence of a multiple

pregnancy [12,13]. We further analyzed the risk factors for twin pregnancies. Petanovski *et al.* [14] demonstrated that increased BMI and older age had negative impacts on pregnancy outcomes. High serum FSH was associated with low-quality oocyte formation and a reduced pregnancy rate [15]. Martin *et al.* [16] indicated it existed a significant negative correlation between gonadotrophin requirements and pregnancy rates. Ovarian stimulation was considered as a risk factor for poor clinical outcomes. Earlier studies indicated that it could impact ideal delivery rates after fresh transfers when the number of retrieved oocytes was between 6 and 15 [17,18]. Nevertheless, we observed that none of these risk factors was independently associated with twin pregnancies in the IVFD-ETs.

Multiple births could be effectively reduced by transferring a single blastocyst [19]. It carried a risk of absent blastocyst formation if extended culture to blastocyst-stage was performed. The blastocyst formation could be affected by various factors. Janny *et al.* [20] showed that the blastulation rate decreased due to a high incidence of embryo arrest at morula stage in patients above age 30. Meanwhile, the number of high-quality cleavage-stage embryos was also one of the key determinants of achieving the ideal number of blastocysts. Thus, it was worth considering the optimal number of day 3 high-quality embryos for blastocyst transfer in the IVFD cycles.

It has been reported that the blastocyst transfers were associated with high implantation rate and low multiple births rate [21]. Nevertheless, it was difficult to predict that the blastocysts could be formed. Rijnders *et al.* [22] concluded that evaluation of day 3 embryo morphology had limited predictive value for subsequent blastocyst formation. It has been reported that the blastulation rate was impaired when the embryo morphology was compromised on day 3 [22]. Braga *et al.* [23] demonstrated that transferring poor cleavage-stage embryos was a better option than transferring embryos at the blastocyst-stage. Thus, it should be careful in recommending the strategy of blastocyst culture for some patients.

Our data showed that the cycle rate of poor quality blastocyst obtained was beyond 30% for cycles with only two good D3 embryos. For such patients, extended culture and blastocyst-stage embryo transfers had negative effects on the clinical outcomes compared with cleavage-stage embryo transfers, although the twin pregnancy rate was significantly decreased. We observed that the clinical outcomes were comparable either by transferring single cleavage-stage high-quality embryo or culturing the embryos to D5 for transfer with two good D3 embryos. For cycles with ≥ 3 high-quality D3 embryos, transferring embryos on D5 were associated with ideal clinical outcomes and significantly decreased the incidence of multiple births.

In conclusion, for patients with ≥ 3 day 3 high-quality embryos, blastocyst transfers were recommended in the IVFD-ETs. For patients with only two day 3 high-quality embryos, single day 3 high-quality embryo transfer was recommended and surplus embryos underwent prolonged cultured to blastocyst-stage. We suggest that a prospective study is needed to further confirm our findings.

5. Conclusions

Our study demonstrated that transferring double cleavage-stage embryos had a high risk of twin pregnancies in the IVFD-ETs. Blastocyst transfer was safe and recommended for the patients with three or more high-quality embryos on day 3.

Abbreviations

IVFD, *in vitro* fertilization with donor sperm; ETs, embryo transfers; BMI, body mass index; FSH, follicle-stimulating hormone; Gn, gonadotropin; CI, confidence interval; OR, odds ratio.

Availability of Data and Materials

Data is available on request corresponding author due to privacy and ethical restrictions.

Author Contributions

ML and JS designed the research. ML and XX performed the research. XX analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This research was approved by the ethics committee of Northwest Women's and Children's Hospital (No. 2022007). Patient consent was not required due to the retrospective nature of the study.

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Conflict of Interest

The authors declare no conflict of interest.

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