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Original Research

The Effects of Number and Quality of Transferred Blastocysts on Birth Outcomes in Frozen-Thawed Transfer Cycles

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Abstract

Background: There are few studies evaluating the effects of number and quality of transferred blastocysts on birth outcomes in frozen-thawed transfer cycles. **Methods**: A retrospective study was conducted, encompassing 5493 frozen-thawed blastocyst transfer cycles from January 2019 to June 2021. The cycles were categorized into five groups based on the number and quality of transferred blastocysts, as well as trichotomized based on maternal age brackets. Pregnancy outcomes such as implantation rate (IR), clinical pregnancy rate (CPR), multiple pregnancy rate (MPR), abortion rate (AR), live birth rate (LBR), and neonatal characteristics were compared and statistically analyzed. **Results**: The data revealed that maternal age, quality and number of the transferred blastocysts exerted a demonstrable impact on both pregnancy and birth outcomes. Within the same blastocyst transfer groups, it was noted that IR, CPR, and LBR exhibited a progressive decline as a function of advancing maternal age. Amplifying the number of homogeneously graded blastocysts for transfer did not conspicuously elevate CPR and LBR; however, it led to a statistically significant escalation in MPR ($p < 0.01$). In instances of dual blastocyst transfers, better-quality blastocysts yielded higher IR, CPR, MPR and LBR. Furthermore, neonatal outcomes were most favorable in singleton births, followed in sequence by dizygotic twins and monozygotic twins. A positive correlation was observed between sex ratio and the proportion of good-quality blastocysts, with a statistically significant difference between good-quality and poor-quality blastocyst groups (1.34 *vs* 1.00, *p*/odds ratio (OR)/95% confidence interval (95% CI) <0.01/1.33/1.10–1.62). **Conclusions**: Single blastocyst transfer appears to be an efficacious strategy for decreasing MPR while achieving favorable pregnancy and birth outcomes. Nonetheless, it should be noted that this strategy may engender a skewed sex ratio among the neonates.

Keywords: age; birth outcomes; blastocyst transfer; multiple pregnancy; neonatal characteristics

1. Introduction

With the development of assisted reproductive technologies (ART), particularly in the domains of embryo culture and cryopreservation, the implantation rate (IR) is increasing along with the multiple pregnancy rate (MPR). Multiple pregnancies were commonly regarded as the most consequential adverse outcomes correlated with ART, as they are associated with elevated risks of maternal and neonatal morbidity. Although, the reduction in the number of transferred embryos has been posited as a critical strategy for mitigating multiple pregnancies, this strategy has not been widely used in clinical practice owing to apprehensions regarding diminished pregnancy rates [1]. Contrary to cleavage-stage embryos, extending embryo culture to the blastocyst stage allowed for better evaluation of the implantation potential, yielding a higher IR [2–4]. Previous studies have posited that elective single blastocyst transfer could yield comparable clinical pregnancy rates (CPR) for patients with a good prognosis [1,5,6]. Although live birth rate (LBR) equivalence was not demonstrated, it was thought the additional complications associated with multiple gestations outweighed the potentially higher LBR [5]. Additionally, frozen-thawed single blastocyst transfers have been found to result in enhanced CPR relative to fresh single blastocyst transfers in ovulatory women with a good prognosis [6]. In the context of advanced maternal age (≥40 years), both single and double blastocyst transfers yielded similar CPR and LBR, while MPR was lower for single blastocyst transfers [7]. Despite burgeoning research in the domain of different embryo-stage and fresh *vs* frozen embryo transfers [8,9], a comprehensive analysis examining the impact of blastocyst number, quality and maternal age on pregnancy and birth outcomes remains lacking. The present study aims to fill this gap by analyzing these variables to formulate a more effective blastocyst transfer strategy for decreasing MPR while achieving desirable pregnancy and birth outcomes.

2. Materials and Methods

2.1 Participants and Study Design

This retrospective study was conducted from January 2019 to June 2021, and focused exclusively on the first frozen-thawed blastocyst transfer cycle. Participants with a diagnosis of either congenital or acquired uterine anomalies, such as uterine malformation, adenomyosis, submucous myoma, uterine fibroids, or intrauterine adhesions were excluded. The cycles were partitioned into five groups based on the number and quality of transferred blastocysts: a single good-quality blastocyst (G), two good-quality blastocysts (GG), a good-quality blastocyst and a poor-quality blastocyst (GP), a single poor-quality blastocyst (P), and two poor-quality blastocysts (PP). Subsequent categorization occurred according to maternal age brackets: $<$35, 35–39 and $>$39 years. Assessed pregnancy and birth outcomes included IR, CPR, MPR, abortion rate (AR), LBR, gestational age, delivery modality, sex ratio, congenital malformation, and birthweight. Abortion was defined as either early or mid-term pregnancy termination, and live birth was construed as the delivery of a live neonate at or beyond a gestational age of 24 weeks.

2.2 Embryo Culture, Freezing and Thawing Protocols

Embryo culture was performed in Quinn's IVF sequential medium suite (Quinn's, SAGE, New York, NY, USA) after adding 10% human serum substitute (Quinn's, SAGE, USA). The atmospheric conditions were precisely regulated to include 5% O_2, 5% CO_2, and 90% N_2, along with a saturated humidity level. Embryos were individually cultured in 25 µL microdroplets of cleavage medium covered with oil. Approximately 3 embryos were co-cultured in each 30 µL microdroplet of blastocyst medium. Blastocysts were scored according to the criteria proposed by Gardner on the morning of day 5 and 6 [10]. Good-quality blastocysts were given a numerical score ranging from 3 to 6 according to their degree of expansion and hatching status. Concurrently, the inner cell mass and trophectoderm were qualitatively assessed and categorized as 'A' or 'B'. In contrast, blastocysts not meeting these rigorous criteria were designated as being of poor-quality. Blastocysts were vitrified and thawed using vitrification media (Kitazato Biopharma, Shizuoka, Japan) and thawed blastocysts were cultured about 2 hours for transfer. The blastocysts were not genetically tested, so the transfers were only based on morphology.

2.3 Endometrial Preparation

Two primary protocols were employed for endometrial preparation: natural and artificial cycles. In natural cycles, either administration of human chorionic gonadotropin (HCG) guided the transfer planning, or a spontaneous luteinizing hormone (LH) peak was detected, with blastocysts transfers occurring on the fifth day post-ovulation. In artificial cycles, a daily oral dose of 3.75 mg commenced on days 2–3 of the menstrual cycle, with dose adjustment made in accordance with the endometrial thickness as gauged by ultrasound. Upon reaching an endometrial thickness of $\geq$7 mm, 40 mg progesterone was injected five days prior to the frozen blastocyst transfer. Luteal phase support was extended until the 11th week of gestation if pregnancy was confirmed.

2.4 Statistical Analyses

Statistical analyses were executed utilizing SPSS version 22.0 (IBM, Armonk, NY, USA). Quantitative variables were presented as means $\pm$ standard deviations, while categorical variables were expressed as frequencies and percentages. A variety of statistical tests including Chi-squared test, Fisher's exact test, T-test and one-way analysis of variance were employed as appropriate. Logistic regression modeling was deployed to probe potential risk factors influencing LBR. A two-sided p-value of $<$0.05 was considered to be statistically significant.

3. Results

3.1 Patient and Cycle Characteristics

The present study encompassed a total of 5493 first frozen-thawed blastocyst transfer cycles. The maternal age, duration of infertility, endometrial thickness and body-mass index were 20–45 years, 0–25 years, 8–14 mm and 13–42 kg/m^2 respectively. The detailed cycle characteristics of the different groups were shown in Table 1. Body-mass index varied significantly in the $<$35 and total age group ($p <$ 0.05). The differences of duration of infertility and primary infertility were significant in the 35–39 and total age group ($p < 0.05$).

3.2 Pregnancy Outcomes

Date presented in Table 2 showed that both the quality and number of transferred blastocysts exerted a palpable impact on pregnancy outcomes. In total age group, IR (74.5%) was observed to be highest in group G, and MPR was the highest in group GG (56.8%) followed by groups GP (36.3%) and PP (29.6%). Additionally, elevated AR was observed in groups P (23.5%) and PP (23.6%). Groups with good-quality blastocysts (groups G, GG, and GP) had higher CPR and LBR alongside diminished AR relative to their counterparts devoid of good-quality blastocysts (groups P and PP). In the groups with good-quality blastocysts, Group G demonstrated a higher IR (p/odds ratio (OR)/95% confidence interval (95% CI) $<$0.01/1.96/1.67–2.30 and $<$0.01/2.73/2.41–3.09) and a lower MPR (p/OR/95% CI $<$0.01/0.02/0.02–0.03 and $<$0.01/0.05/0.04–0.06) in comparison to groups GG and GP. Meanwhile, CPR, AR, and LBR were similar among these groups ($p > 0.05$). The CPR, AR, and LBR were similar ($p > 0.05$) between groups GG and GP, while MPR was higher in group GG (p/OR/95% CI $<$0.01/2.31/1.75–3.04). In contrast, between the groups without good-quality

Table 1. Patient and cycle characteristics.

Age (years)	Embryo	Body-mass index (kg/m^2)	Natural cycle (%)	Endometrial thickness (mm)	Duration of infertility (years)	Primary infertility (%)
<35	G	21.3 ± 2.6	6.7 (163/2416)	9.5 ± 0.9	3.5 ± 2.4	43.0 (1039/2416)
	GG	21.7 ± 2.7	5.4 (15/277)	9.5 ± 0.9	3.7 ± 2.7	47.7 (132/277)
	GP	21.5 ± 2.5	8.4 (39/464)	9.5 ± 0.9	3.7 ± 2.6	45.7 (212/464)
	PP	21.1 ± 2.4	5.6 (18/320)	9.5 ± 1.2	3.6 ± 2.6	47.8 (153/320)
	P	21.6 ± 2.6	8.9 (10/112)	9.6 ± 1.0	3.1 ± 2.0	38.4 (43/112)
	p-value	0.03	0.38	0.94	0.13	0.18
35–39	G	22.0 ± 2.5	11.6 (94/813)	9.6 ± 1.1	4.5 ± 3.4	17.5 (142/813)
	GG	21.8 ± 2.5	13.8 (16/116)	9.8 ± 1.1	4.6 ± 3.6	20.7 (24/116)
	GP	22.3 ± 3.0	9.4 (24/256)	9.8 ± 1.0	4.9 ± 3.5	20.7 (53/256)
	PP	22.1 ± 2.8	12.3 (30/243)	9.8 ± 1.2	5.1 ± 3.8	26.3 (64/243)
	P	22.0 ± 2.6	12.0 (13/108)	9.5 ± 1.2	4.0 ± 2.9	13.9 (15/108)
	p-value	0.46	0.72	0.09	0.03	0.02
>39	G	22.6 ± 2.6	18.2 (22/121)	9.4 ± 1.4	5.9 ± 4.9	11.6 (14/121)
	GG	22.2 ± 2.1	14.8 (4/27)	9.6 ± 1.0	3.9 ± 4.2	11.1 (3/27)
	GP	22.6 ± 2.4	15.2 (12/79)	9.4 ± 1.1	5.4 ± 5.1	11.4 (9/79)
	PP	22.2 ± 2.4	13.0 (13/100)	9.5 ± 1.4	6.7 ± 5.9	13.0 (13/100)
	P	22.7 ± 3.5	19.5 (8/41)	9.3 ± 1.1	5.4 ± 3.8	14.6 (6/41)
	p-value	0.72	0.81	0.82	0.09	0.98
Total	G	21.5 ± 2.6	8.3 (279/3350)	9.6 ± 1.0	3.9 ± 2.9	35.7 (1195/3350)
	GG	21.8 ± 2.6	8.3 (35/420)	9.6 ± 1.0	4.0 ± 3.1	37.9 (159/420)
	GP	21.9 ± 2.7	9.4 (75/799)	9.6 ± 1.0	4.3 ± 3.3	34.3 (274/799)
	PP	21.7 ± 2.6	10.7 (71/663)	9.6 ± 1.2	4.6 ± 3.9	34.7 (230/663)
	P	21.9 ± 2.8	11.9 (31/261)	9.5 ± 1.3	3.8 ± 2.8	24.5 (64/261)
	p-value	<0.01	0.12	0.57	<0.01	<0.01

Note: G, a good-quality blastocyst; GG, two good-quality blastocysts; GP, a good-quality blastocyst and a poor-quality blastocyst; PP, two poor-quality blastocysts; P, a poor-quality blastocyst.

blastocysts, group P similarly outperformed group PP in terms of both higher IR (*p*/OR/95% CI <0.01/1.63/1.25–2.12) and lower MPR (*p*/OR/95% CI <0.01/0.06/0.02–0.18), while CPR, AR, and LBR did not manifest significant differences ($p > 0.05$). These results indicated that increasing the number of same grade blastocysts transferred did not obviously increase CPR and LBR, but rather markedly elevated MPR. Concerning double blastocyst transfer scenarios (groups GG, GP and PP), a superior blastocyst grade corresponded to heightened IR, CPR, MPR, and LBR. These rates exhibited a pronounced decrement in group PP (GG *vs* PP, *p*/OR/95% CI <0.01/2.37/1.99–2.83, <0.01/2.19/1.66–2.88, <0.01/3.12/2.30–4.25 and <0.01/1.98/1.54–2.54 respectively). For single blastocyst transfer, group G evidenced elevated IR, CPR, and LBR (*p*/OR/95% CI <0.01/2.86/2.22–3.69, <0.01/2.86/2.22–3.69, <0.01/2.60/2.01–3.37, respectively) and diminished AR (*p*/OR/95% CI = 0.42/0.64/0.42–0.97) in comparison to group P.

In different age groups, a progressive attenuation in IR, CPR, and LBR was discernible with advancing maternal age, reaching statistical significance beyond 39 years ($p < 0.05$). The MPR in groups GG, GP, and PP also demonstrated a corresponding decline with increasing age ($p < 0.01$). In comparison to the group <35, the AR was significantly elevated in group >39 ($p < 0.05$). Across all age classifications, the trends in IR, CPR, MPR, AR, and LBR remained congruent with those observed in the total age group.

Logistic regression modeling, delineated in Table 3, incorporated variables that were both statistically significant according to Table 1 and clinically salient. This model indicated that the most potent predictors of live birth were maternal age and transferred blastocysts. The LBR within cohorts of identically graded blastocyst transfers exhibited a gradual diminution with increasing age. Cohorts comprising good-quality blastocysts (groups G, GG and GP) consistently outperformed those bereft of such blastocysts (groups PP and P) in terms of LBR (*p*/OR/95% CI <0.01/2.13/1.84–2.45).

3.3 Neonatal Characteristics

Tables 4,5 offered a meticulous portrayal of neonatal attributes for live-born singletons and twins. Comparative analyses revealed that the singleton group had a higher average gestational age (*p*/OR/95% CI <0.01/2.66/2.43–2.88) and birthweight (*p*/mean difference (MD)/95% CI <0.01/818.38/772.70–864.05) as well as a lower cesarean

Table 2. The effects of quality and number of transferred blastocysts on pregnancy outcomes.

Age (years)	Embryo	IR	CPR	MPR	AR	LBR
<35	G	78.4 (1893/2416)$^{a}{}_{A}$	78.4 (1893/2416)$^{a}{}_{A}$	2.7 (52/1893)$^{c}{}_{A}$	13.7 (259/1893)$^{ba}{}_{C}$	67.5 (1630/2416)$^{a}{}_{A}$
	GG	65.9 (365/554)$^{b}{}_{A}$	78.7 (218/277)$^{a}{}_{A}$	67.4 (147/218)$^{a}{}_{A}$	11.0 (24/218)$^{b}{}_{C}$	70.0 (194/277)$^{a}{}_{A}$
	GP	56.7 (526/928)$^{c}{}_{A}$	80.4 (373/464)$^{a}{}_{A}$	41.0 (153/373)$^{b}{}_{A}$	11.5 (43/373)$^{b}{}_{B}$	70.9 (329/464)$^{a}{}_{A}$
	PP	45.8 (293/640)$^{d}{}_{A}$	68.4 (219/320)$^{b}{}_{A}$	34.2 (75/219)$^{b}{}_{A}$	20.1 (44/219)$^{a}{}_{B}$	54.7 (175/320)$^{b}{}_{A}$
	P	58.9 (66/112)$^{bc}{}_{A}$	58.9 (66/112)$^{b}{}_{A}$	3.0 (2/66)$^{c}{}_{A}$	22.7 (15/66)$^{ab}{}_{A}$	45.5 (51/112)$^{b}{}_{A}$
35–39	G	66.5 (541/813)$^{a}{}_{B}$	66.5 (541/813)$^{ab}{}_{B}$	1.8 (10/541)$^{b}{}_{A}$	23.7 (128/541)$^{ab}{}_{B}$	50.7 (412/813)$^{ab}{}_{B}$
	GG	50.4 (117/232)$^{b}{}_{B}$	75.0 (87/116)$^{a}{}_{A}$	35.6 (31/87)$^{a}{}_{B}$	31.0 (27/87)$^{a}{}_{A}$	51.7 (60/116)$^{ab}{}_{B}$
	GP	45.7 (234/512)$^{b}{}_{B}$	69.9 (179/256)$^{ab}{}_{B}$	31.8 (57/179)$^{a}{}_{AB}$	17.9 (32/179)$^{b}{}_{B}$	57.4 (147/256)$^{a}{}_{B}$
	PP	36.8 (179/486)$^{c}{}_{B}$	59.3 (144/243)$^{bc}{}_{A}$	26.4 (38/144)$^{a}{}_{AB}$	20.8 (30/144)$^{ab}{}_{B}$	46.9 (114/243)$^{ab}{}_{A}$
	P	50.9 (55/108)$^{b}{}_{A}$	50.9 (55/108)$^{c}{}_{A}$	1.8 (1/55)$^{b}{}_{A}$	20.0 (11/55)$^{ab}{}_{A}$	40.7 (44/108)$^{b}{}_{A}$
>39	G	52.1 (63/121)$^{a}{}_{C}$	52.1 (63/121)$^{a}{}_{C}$	4.8 (3/63)$^{b}{}_{A}$	36.5 (23/63)$^{a}{}_{A}$	33.1 (40/121)$^{a}{}_{C}$
	GG	38.9 (21/54)$^{ac}{}_{B}$	63.0 (17/27)$^{a}{}_{A}$	29.4 (5/17)$^{a}{}_{B}$	47.1 (8/17)$^{a}{}_{A}$	33.3 (9/27)$^{a}{}_{B}$
	GP	42.4 (67/158)$^{a}{}_{B}$	70.9 (56/79)$^{a}{}_{B}$	19.6 (11/56)$^{a}{}_{B}$	46.4 (26/56)$^{a}{}_{A}$	38.0 (30/79)$^{a}{}_{C}$
	PP	20.0 (40/200)$^{b}{}_{C}$	35.0 (35/100)$^{b}{}_{B}$	14.3 (5/35)$^{ab}{}_{B}$	57.1 (20/35)$^{a}{}_{A}$	15.0 (15/100)$^{b}{}_{B}$
	P	26.8 (11/41)$^{bc}{}_{B}$	26.8 (11/41)$^{b}{}_{B}$	0.0 (0/11)$^{ab}{}_{A}$	45.5 (5/11)$^{a}{}_{A}$	14.6 (6/41)$^{b}{}_{B}$
Total	G	74.5 (2497/3350)^{a}	74.5 (2497/3350)^{a}	2.6 (65/2497)^{d}	16.4 (410/2497)^{b}	62.1 (2082/3350)^{a}
	GG	59.9 (503/840)^{b}	76.7 (322/420)^{a}	56.8 (183/322)^{a}	18.3 (59/322)^{ab}	62.6 (263/420)^{a}
	GP	51.8 (827/1598)^{c}	76.1 (608/799)^{a}	36.3 (221/608)^{b}	16.6 (101/608)^{bc}	63.3 (506/799)^{a}
	PP	38.6 (512/1326)^{d}	60.0 (398/663)^{b}	29.6 (118/398)^{c}	23.6 (94/398)^{a}	45.9 (304/663)^{b}
	P	50.6 (132/261)^{bc}	50.6 (132/261)^{c}	2.3 (3/132)^{d}	23.5 (31/132)^{ac}	38.7 (101/261)^{b}

Note: Superscript lowercase letters (a, b, c) demonstrate the differences of different transferred blastocysts in the same age groups, while subscript uppercase letters (A, B, C) signify differences of the same transferred blastocysts in the different age groups. Completely different letters indicate a significant difference ($p < 0.05$), and any of the same letters are not significant ($p > 0.05$). IR, implantation rate; CPR, clinical pregnancy rate; MPR, multiple pregnancy rate; AR, abortion rate; LBR, live birth rate.

Table 3. Logistic regression analysis of the risk factors affecting the live birth rate.

	Wals	*p*	OR (95% CI)
Blastocysts			
P	64.60	<0.01	1.00
G	26.68	<0.01	2.02 (1.55–2.64)
GG	21.58	<0.01	2.17 (1.56–3.01)
GP	37.43	<0.01	2.51 (1.87–3.37)
PP	2.90	0.09	1.30 (0.96–1.75)
Endometrial thickness	0.69	0.41	1.02 (0.97–1.08)
Female age	151.92	<0.01	0.91 (0.90–0.93)
Duration of infertility	1.85	0.17	0.99 (0.97–1.01)
Body-mass index	0.33	0.57	1.01 (0.99–1.03)
Type of infertility	3.22	0.07	0.89 (0.79–1.01)
Endometrial preparation	2.77	0.10	1.18 (0.97–1.43)

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

section rate (*p*/OR/95% CI <0.01/0.10/0.06–0.15), preterm labor rate (*p*/OR/95% CI <0.01/0.06/0.05–0.08) and low birthweight rate (*p*/OR/95% CI <0.01/0.06/0.05–0.07) than the twin group. Moreover, no significant differences were present in parameters such as sex ratio and congenital malformation rate between the two groups (*p*/OR/95% CI = 0.39/1.07/0.91–1.26 and 0.50/0.81/0.43–1.52). Table 5 illustrated that blastocyst transfer strategies exerted a significant impact on pivotal neonatal outcomes, such as gestational age, cesarean section and birthweight in the singleton group ($p < 0.01$). When focusing on twin births, Monozygotic twins displayed a significantly reduced gestational age (34.83 $\pm$ 2.89 *vs* 35.90 $\pm$ 2.04, *p*/MD/95% CI <0.01/–1.07/–1.82–0.32) and birthweight (2225.00 $\pm$ 492.20 *vs* 2481.52 $\pm$ 403.21, *p*/MD/95% CI <0.01/–256.53/–402.04–111.03) in comparison to their dizygotic counterparts. Concurrently, monozygotic twins manifested an elevated incidence of both preterm birth rate (76.47% *vs* 56.94%, *p*/OR/95% CI = 0.03/2.46/1.08–5.58) and low birthweight rate (66.18% *vs* 44.34%, *p*/OR/95% CI <0.01/2.45/1.45–4.15). Remarkably, an intriguing pattern emerged concerning the sex ratio across different blastocyst quality classifications. The good-quality blastocyst groups (groups G and GG) (1.34, 1436/1073) exhibited the highest sex ratio, followed by the moderate-quality blastocyst group (GP) (1.17, 350/298) and poor-quality blastocyst groups (groups P and PP) (1.00, 240/239). A statistically significant discrepancy was discerned between the sex ratios of good-quality and poor-quality blastocyst groups (*p*/OR/95% CI <0.01/1.33/1.10–1.62).

4. Discussion

Pertaining to the total age group under study, group G exhibited the highest IR, the lowest MPR, and analogous CPR, AR, and LBR when compared to other cohorts em-

Table 4. Pregnancy outcomes of live born singletons and twins.

Characteristics	Singleton (n = 2876)	Twin (n = 380)	*p*	OR/MD (95% CI)
Gestational age (weeks)	38.5 ± 1.7	35.8 ± 2.15	<0.01	2.66 (2.43–2.88)
<32	0.8% (24)	4.2% (16)	<0.01	0.19 (0.10–0.36)
<37	8.2% (236)	58.7% (223)	<0.01	0.06 (0.05–0.08)
≥37	91.8% (2640)	41.3% (157)	<0.01	15.89 (12.45–20.28)
Birthweight (g)	3276.9 ± 484.0	2458.57 ± 417.69	<0.01	818.38 (772.70–864.05)
<1500	0.6% (18)	2.8% (21)	<0.01	0.22 (0.12–0.42)
<2500	4.6% (133)	46.3% (352)	<0.01	0.06 (0.05–0.07)
≥2500	95.4% (2743)	53.7% (408)	<0.01	17.79 (14.21–22.28)
Cesarean section	61.9% (1779)	94.5% (359)	<0.01	0.10 (0.06–0.15)
Sex ratio (male/female)	1.3 (1613/1263)	1.2 (413/347)	0.39	1.07 (0.91–1.26)
Congenital malformations	1.4 % (40)	1.7% (13)	0.50	0.81 (0.43–1.52)

Note: MD, mean difference.

Table 5. Pregnancy outcomes of live born singletons and twins in different blastocyst transfer groups.

Characteristics		G	GG	GP	PP	P	*p*
Gestational age (weeks)	S	38.6 ± 1.6	38.2 ± 1.7	38.2 ± 2.1	38.3 ± 1.8	38.0 ± 1.8	<0.01
	T	34.9 ± 3.0	35.8 ± 2.1	35.8 ± 2.0	36.2 ± 2.0	34.5 ± 0.7	0.06
<37 weeks	S	7.0% (144/2050)	14.5% (19/131)	9.6% (35/364)	10.8% (25/232)	13.1% (13/99)	<0.01
	T	75.0% (24/32)	61.4% (81/132)	57.8% (82/142)	47.2% (34/72)	100.0% (2/2)	0.05
Cesarean section	S	59.4% (1217/2050)	67.9% (89/131)	65.7% (239/364)	68.1% (158/232)	76.8% (76/99)	<0.01
	T	87.5% (28/32)	94.7% (125/132)	95.1% (135/142)	95.8% (69/72)	100.0% (2/2)	0.44
Birthweight (g)	S	3296.3 ± 468.5	3223.7 ± 489.3	3247.8 ± 539.0	3225.5 ± 494.49	3173.8 ± 529.5	0.01
	T	2230.5 ± 506.0	2469.7 ± 370.2	2499.0 ± 399.8	2468.9 ± 467.7	2137.5 ± 123.7	0.02
Birthweight <2500	S	4.1% (84/2050)	8.4% (11/131)	4.9% (18/364)	4.7% (11/232)	9.1% (9/99)	0.04
	T	64.1% (41/64)	43.9% (116/264)	45.8% (130/284)	42.4% (61/144)	100.0% (4/4)	0.01
Sex ratio (male/female)	S	1.33 (1171/879)	1.43 (77/54)	1.19 (198/166)	1.02 (117/115)	1.02 (50/49)	0.20
	T	1.06 (33/31)	1.42 (155/109)	1.15 (152/132)	0.92 (69/75)	4.00 (4/0)	0.09
Sex ratio (male/female)	Total	1.34 (1436/1073)		1.17 (350/298)	1.00 (240/239)		0.01
Congenital malformations	S	1.3% (27/2050)	0.0% (0/131)	2.2% (8/364)	1.7% (4/232)	1.0% (1/99)	0.41
	T	4.7% (3/64)	0.8% (2/264)	2.1% (6/284)	1.4% (2/144)	0.0% (0/4)	0.20

Note: S, singleton; T, twin. The differences of gestational age, cesarean section rate, birthweight, preterm labor rate and low birthweight rate were significant ($p < 0.01$), when group singleton *vs* twin is in the same embryo transfer group.

ploying good-quality blastocysts. Likewise, group P had a higher IR, lower MPR, and similar CPR, AR and LBR in relation to group PP, which is in consonance with previous studies [11–14]. In regard to double blastocyst transfer, the MPR of groups GG, GP and PP were 56.8%, 36.3% and 29.6%, respectively. Importantly, these trends across diverse age subgroups were consistent with those observed in the total age group. Consequently, the obtained results indicated that single blastocyst transfer appeared to be an efficacious strategy for minimizing MPR while achieving favorable LBR. The logistic regression analysis further accentuated that maternal age and transferred blastocysts were factors significantly correlated with LBR. Specifically, within the age group of <35, the MPRs for groups GG, GP, and PP were 67.4%, 41.0% and 34.2%, respectively. Hence, our findings implicitly advocate for the utilization of single blastocyst transfer strategies over their double blastocyst counterparts, particularly for younger patients. Notably, our analyses also revealed that increasing the number of transferred blastocysts of the same grade did not engender a substantial elevation in CPR and LBR but did significantly increase MPR. The results were similar to those of previous studies [12,15,16]. One limitation that merits acknowledgment is that our study classified blastocyst quality only dichotomously, potentially introducing variance within quality levels that could have nuanced implications for pregnancy outcomes.

Pregnancy is often conceptualized as a nuanced interplay, mediated by localized secretion of key factors, between a developmentally competent embryo and a recep-

tive endometrium. Existing research posits that decidualized human endometrial stromal cells possess the ability to selectively identify developmentally impaired embryos and respond by inhibiting the secretion of key implantation mediators such as Interleukin-1β (IL-1β) and heparin-binding EGF-like growth factor (HB-EGF), as well as immunomodulators including IL-5, -6, -10, -11, -17, and eotaxin [17]. Impaired embryos elicited an endoplasmic stress response in human decidual cells. Conversely, signals originating from developmentally competent embryos activated a focused gene network enriched in metabolic enzymes and implantation factors [18]. Moreover, high-quality embryos have been observed to incite transient, oscillatory Ca21 fluxes, whereas low-quality embryos induce an elevated and prolonged Ca21 response [18]. These dichotomous mechanisms may contribute to diminished IR among poor-quality blastocysts. This inhibitory phenomenon might be cumulative, thereby further depressing the IR when two low-quality blastocysts were transferred simultaneously. A solitary good-quality blastocyst has been noted to yield a higher IR compared to the transfer of two such blastocysts, potentially due to insufficient secretion of critical implantation factors in the latter scenario. Our observations with regard to IRs in age groups <35 and 35–39, as well as the broader patient demographic, echo existing literature, indicated that a reduced IR for GP (one good-quality and one poor-quality blastocyst) might be attributed to biochemical reactions that obstructed the implantation of the lower-quality blastocyst, consequently affecting the implantation potential of its higher-quality blastocyst [12,14]. Moreover, our data showed credence to the hypothesis that competitive interactions might exist between co-transferred blastocysts, manifesting as compromised IR for double blastocyst transfers. Previous findings had demonstrated that IRs were high in the following order: single good embryo, double good embryos, one good embryo with a poor embryo, single bad embryo and double bad embryos [14]. Blastocyst score and proportion of top-scoring blastocyst affected implantation, and the degree of blastocoele re-expansion have been affirmed as potent predictors of live birth outcomes in warmed single blastocyst transfer cycles [10,19], corroborating the findings of our study.

Multiple pregnancy is considered the most significant adverse event associated with ART and linked to an increased risk of maternal and neonatal morbidity. The results indicated that single blastocyst transfer had a lower MPR (2.6% *vs* 39.3%, *p*/OR/95% CI <0.01/0.04/0.03–0.05), AR (16.8% *vs* 19.1%, *p*/OR/95% CI = 0.07/0.85/0.72–1.01), cesarean section rate (60.6% *vs* 76.0%, *p*/OR/95% CI <0.01/0.49/0.41–0.57), preterm labor rate (8.4% *vs* 25.7%, *p*/OR/95% CI <0.01/0.26/0.22–0.32) and ectopic pregnancy rate (0.38% *vs* 1.81%, *p*/OR/95% CI <0.01/0.21/0.10–0.44) than two blastocyst transfer, while the singleton group had a higher average gestational age and birthweight as well as a lower birthweight rate. Importantly, LBR were observed to be statistically analogous between single and double blastocyst transfers, irrespective of blastocyst quality categorizations. Consequently, the implementation of single blastocyst transfer strategies is advocated as a potent measure for mitigating the associated morbidity risks intrinsic to multiple pregnancies. Intriguingly, our study delineated distinct health markers between monozygotic and dizygotic twins. The former had a lower gestational age and birthweight in conjunction with a higher preterm birth rate and low birthweight rate. Therefore, monozygotic twins deserved more attention in the context of ART treatments.

In vitro culture has been shown to induce precocious X-chromosome inactivation, and intracytoplasmic sperm injection (ICSI) is implicated in reducing the number of trophectoderm cells in female blastocysts [20]. Sex ratio was significantly higher toward males in the transfer of blastocyst compared to transfer of cleavage stage embryo [20–23]. Our data further substantiate a significant positive correlation between sex ratio (male/female) and the proportion of good-quality blastocysts transferred. As, the practice of blastocyst culture and selective single good-quality blastocyst transfer gains scholarly endorsement [6,12,24], the potential for such strategies to engender imbalances in neonatal sex ratios remains an emergent area requiring further research.

5. Conclusions

Our empirical analyses confirm the efficacy of single blastocyst transfer as an optimal strategy for significantly attenuating MPR while ensuring favorable pregnancy and birth outcomes. Nonetheless, it should be noted that this strategy may engender a skewed sex ratio among the neonates. Furthermore, the distinct health metrics of monozygotic twins deserve more attention in the context of ART treatments.

Availability of Data and Materials

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

Author Contributions

The study was designed by YL and BM. LZ, PY, NL and YL were involved in planning and managing the data collection. YL and BM were involved in the statistical analysis and wrote the manuscript with support. Critical review was from all authors. 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

Ethical approval was granted by the Ethics Committee of Haikou Mary Hospital on the March 8, 2020 (NO. 02/MLYY/2020). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

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Conflict of Interest

The authors declare no conflict of interest.

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