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# The risk of birth defects among children born after vitrified blastocyst transfers and those born after fresh and vitrified cleavage-stage embryo transfers

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## Abstract

**Purpose** To explore the risk of birth defects among children born after vitrified blastocyst transfers and those born after fresh and vitrified cleavage-stage embryo transfers.

**Methods** A retrospective cohort study was conducted including infants born after fresh and vitrified day 3 embryo transfers and those born after vitrified day 5 or 6 blastocyst transfers from January 2005 through December 2016. The outcome measures included any birth defect, multiple birth defects and 13 individual categories of birth defects.

**Results** Any birth defect occurred in 1.15% of infants born after fresh day 3 embryo transfers, 1.75% of infants born after vitrified day 3 embryo transfers, 1.60% of infants born after vitrified day 5 blastocyst transfers and 1.10% of infants born after vitrified day 6 blastocyst transfers. There was no difference in the risk of birth defects between vitrified blastocyst-stage transfers and vitrified cleavage-stage transfers (including day 5 vs. day 3 and day 6 vs. day 3) among all births or in only singletons or twins. For infants born after cleavage-stage embryo transfers at day 3, there was no difference in the risk of birth defects between fresh embryo transfers and vitrified embryo transfers among all births or in only singletons or twins.

**Conclusions** Transfer of vitrified day 5 or 6 blastocysts does not increase the risk of birth defects compared with vitrified day 3 embryos. However, randomized control trials and follow-up studies of the long-term outcome of children born after blastocyst-stage transfers are needed to confirm the clinical safety of extending embryo culture to the blastocyst stage.

**Keywords** Birth defects · In vitro fertilization · Blastocyst transfer · Cleavage-stage embryo transfer · Frozen-thawed embryo transfer

## Introduction

Since the first IVF pregnancy after blastocyst transfer was reported, the practice of extended embryo culture to the blastocyst stage, which is regarded as a tool to select the most viable embryos, is increasingly being adopted in assisted reproductive technologies (ART) [1]. Furthermore, an increasing number of studies have focused on neonatal outcomes after blastocyst-stage transfers, though there is no clear evidence supporting its safety [2–6]. Conflicting results have been reported concerning birth defects (also called congenital anomalies or congenital malformations) in infants born after blastocyst vs. cleavage-stage transfers. A study using data from all IVF clinics in Sweden from 2002 to 2006 found that the risk for any birth defect was significantly higher among infants born after blastocyst transfers than cleavage-stage embryo transfers (aOR 1.43, 95% CI 1.14–1.81) [5]. However, a retrospective cohort study performed in Canada from 2001 to 2009 did not find a significant difference in the risk of birth defects among 12,712 singleton births born after blastocyst transfers and cleavage-stage transfers (aOR 1.13, 95% CI 0.85–1.50) [6].

In addition to these controversial results, the previous studies examining birth defects in infants born after blastocyst and cleavage-stage transfers have other important

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limitations: they did not distinguish between fresh and frozen embryo transfers and did not consider the developmental stage of the embryo at transfer for both cleavage stage (day 2 vs. day 3) and blastocysts (day 5 vs. day 6). The objective of our study was to compare the birth defects of singleton and twin infants born after vitrified blastocyst and cleavage-stage embryo transfers (including day 5 vs. day 3 and day 6 vs. day 3) in a large cohort of infants born after ART. In addition, we also compared the risk of birth defects for infants born after fresh cleavage embryo transfers (day 3) with those after vitrified cleavage embryo transfer (day 3).

## Materials and methods

This retrospective cohort study was carried out in China. The data were obtained from the ART database at the Department of Assisted Reproduction of the Shanghai Ninth People's Hospital, affiliated with Jiaotong University, School of Medicine (a large hospital-based tertiary care reproductive center in Shanghai, China), for the period beginning January 2005 through December 2016. Details of treatment with ART and any birth resulting from ART were recorded in this database, which was required by the Technical Standard for Human Assisted Reproduction issued by the Chinese Ministry of Health (CMOH). The patients provided information about their obstetric and perinatal outcomes during a telephone interview conducted within 1 month after delivery.

The details of ovarian stimulation, embryo culture, endometrium preparation and embryo transfer have been described in our previous study [7]. Embryos were graded on the third day according to the Cummins' criteria. All good-quality embryos (including grade 1 and grade 2 8-cell embryos) were transferred or frozen by vitrification on the third day after oocyte retrieval. The non-top-quality embryos were extendedly cultured and observed until they reached the blastocyst stage. At this stage, only good-morphology blastocysts were frozen on days 5 or 6.

The population of this study included all births conceived with the use of ART. A live birth was defined as a birth exhibiting any sign of life, irrespective of the duration of gestation, according to the definition used by the World Health Organization. The details of the assessment for birth defects have been described in a previously published paper [7]. Briefly, neonates born in our hospitals received a routine physical examination at birth, and written health reports were provided by the primary pediatrician for neonates born in other hospitals. For infants with birth defects, an independent pediatrician examined the case reports based on clinical experience to ensure that these infants met the inclusion criteria of the Chinese Birth Defects Monitoring Program. Birth defects were defined and coded according to the International Classification of Diseases, 10th Revision (ICD-10). Minor birth defects were excluded, except those that required treatment or were disfiguring. Thirteen categories of birth defects were coded in this study. In addition, we created two categories for 'any birth defect' and 'multiple birth defects', which accounted for an infant with any of the birth defects or more than one birth defect. The cases with several birth defects were counted as one case in each subgroup, but they could be assigned to more than one subgroup.

This study was approved by the Ethics Committee (Institutional Review Board) of the Shanghai Ninth People's Hospital. All participants provided written informed consent for this study.

## Statistical analysis

All births were classified into four groups: fresh day 3 embryo transfers, vitrified day 3 embryo transfers, vitrified day 5 blastocyst transfers and vitrified day 6 blastocyst transfers. We collected data describing the demographic characteristics of infants in the four groups. The demographic variables included plurality, birth weight, gestational age, maternal age, female infertility type, causes of infertility, maternal body mass index (BMI), type of ART and number of embryos transferred. Subjects were categorized into four groups based on maternal age (< 30 years, 30–34 years, 35–37 years and > 37 years). We computed the percentage for categorical variables and the mean (standard deviation, SD) for quantitative variables. Next, we evaluated the proportion of any birth defect, multiple birth defects and the categories of birth defects among all infants, singletons and twins across the four groups (fresh day 3 embryo transfers, vitrified day 3 embryo transfers, vitrified day 5 blastocyst transfers and vitrified day 6 blastocyst transfers). Logistic regression models were used to compute odds ratios (OR) for estimating the effect of embryo developmental stage at transfer on any birth defects and the categories of birth defects among all infants, singletons and twins. The models were adjusted for maternal age, infertility type, causes of infertility, year of birth, type of cycle (IVF/ICSI) and number of embryos transferred. Results are reported as adjusted odds ratios (aORs) with 95% confidence intervals (CIs).

All statistical analyses were performed using a two-sided 5% level of significance and the statistical package Stata, Version 12.

## Results

In total, 954 infants were born after fresh day 3 embryo transfers, 13,920 infants after vitrified day 3 embryo transfers, 687 infants after vitrified day 5 blastocyst transfers and

1454 infants after vitrified day 6 blastocyst transfers. The proportion of singleton births was 75.37%, 73.58%, 71.18%, and 80.81% respectively, across the above four groups. Table 1 describes the baseline maternal characteristics in the four groups.

The birth defects in all infants are presented in Table 2. Any birth defect occurred in 1.15% of infants born after fresh day 3 embryo transfers, 1.75% of infants born after vitrified day 3 embryo transfers, 1.60% of infants after vitrified day 5 blastocyst transfers and 1.10% of infants after vitrified day 6 blastocyst transfers. The proportion of multiple birth defects was 0.10, 0.10, 0 and 0.07% respectively, across the four groups. When analyzing specific categories of birth defects, the proportion of circulatory system defects was higher than the other categories of birth defects in all four groups (0.31, 0.91, 0.73 and 0.62% respectively), followed by respiratory system, musculoskeletal system, and digestive system defects.

Table 3 presents the proportion of birth defects stratified by singletons and twins. The proportion of any birth defect among singletons was 0.97% in the fresh day 3 embryo transfers group, 1.35% in the vitrified day 3 embryo transfers

**Table 1** Characteristics of births conceived with cleavage-stage or blastocyst-stage transfers in China, 2005–2017

| Characteristic | Fresh day 3 embryo transfers ($n=954$) | Vitrified day 3 embryo transfers ($n=13{,}920$) | Vitrified day 5 blastocyst transfers ($n=687$) | Vitrified day 6 blastocyst transfers ($n=1454$) | $p$ value |
|---|---|---|---|---|---|
| Plurality | | | | | C |
| Singleton | 719 (75.37) | 10,242 (73.58) | 489 (71.18) | 1175 (80.81) | |
| Twin | 235 (24.63) | 3675 (26.40) | 198 (28.82) | 278 (19.12) | |
| Birth weight, g–mean (SD) | 3127.33 (18.66) | 3125.61 (5.14) | 3136.67 (24.89) | 3198.65 (15.84) | C |
| Gestation, week | | | | | B, C |
| <32 | 15 (1.57) | 284 (2.04) | 22 (3.20) | 25 (1.72) | |
| 32–36 | 176 (18.45) | 2511 (18.04) | 168 (24.45) | 298 (20.50) | |
| 37–40 | 747 (78.30) | 10,775 (77.41) | 481 (70.01) | 1110 (76.34) | |
| >40 | 16 (1.68) | 350 (2.51) | 16 (2.33) | 21 (1.44) | |
| Maternal age, year | | | | | A, B |
| < 30 | 376 (39.41) | 4973 (35.73) | 274 (39.88) | 501 (34.46) | |
| 30–34 | 422 (44.23) | 6110 (43.89) | 303 (44.10) | 646 (44.43) | |
| 35–37 | 118 (12.37) | 1837 (13.20) | 78 (11.35) | 215 (14.79) | |
| > 37 | 38 (3.98) | 1000 (7.18) | 32 (4.66) | 92 (6.33) | |
| Female infertility type | | | | | B |
| Primary infertility | 491 (51.47) | 7477 (53.71) | 342 (49.78) | 748 (51.44) | |
| Secondary infertility | 463 (48.53) | 6443 (46.29) | 345 (50.22) | 706 (48.56) | |
| Causes of infertility | | | | | A, B |
| Male only | 105 (11.01) | 1604 (11.52) | 47 (6.84) | 147 (10.11) | |
| Female only | | | | | |
| Endometriosis only | 14 (1.47) | 74 (0.53) | 4 (0.58) | 12 (0.83) | |
| Ovulatory only | 28 (2.94) | 323 (2.32) | 25 (3.64) | 28 (1.93) | |
| Tubal only | 415 (43.50) | 5102 (36.65) | 324 (47.16) | 582 (40.03) | |
| Mixed and other causes | 196 (20.55) | 3071 (22.06) | 139 (20.23) | 300 (20.63) | |
| Male and female combined | 146 (15.30) | 2895 (20.80) | 115 (16.74) | 291 (20.01) | |
| Unknown causes | 50 (5.24) | 851 (6.11) | 33 (4.80) | 94 (6.46) | |
| Type of cycle | | | | | A, B, C |
| IVF | 707 (74.11) | 9483 (68.13) | 556 (80.93) | 1040 (71.53) | |
| ICSI | 247 (25.89) | 4437 (31.87) | 131 (19.07) | 414 (28.47) | |
| Number of embryos transfer | | | | | B, C |
| 1 | 74 (7.76) | 883 (6.34) | 306 (44.54) | 592 (40.72) | |
| 2 | 880 (92.24) | 13,037 (93.66) | 381 (55.46) | 862 (59.28) | |

A: $p<0.05$ for comparisons between births conceived with fresh day 3 embryo transfers and vitrified day 3 embryo transfers

B: $p<0.05$ for comparisons between births conceived with vitrified day 3 embryo transfers and vitrified day 5 blastocyst transfers

C: $p<0.05$ for comparisons between births conceived with vitrified day 3 embryo transfers and vitrified day 6 blastocyst transfers

**Table 2** Proportion for birth defects of all infants conceived with cleavage-stage or blastocyst-stage transfers in China, 2005–2017

| | Fresh day 3 embryo transfers ($n=954$) | Vitrified day 3 embryo transfers ($n=13{,}920$) | Vitrified day 5 blastocyst transfers ($n=687$) | Vitrified day 6 blastocyst transfers ($n=1454$) |
|---|---|---|---|---|
| Any defect | 11 (1.15) | 243 (1.75) | 11 (1.60) | 16 (1.10) |
| Multiple defects | 1 (0.10) | 14 (0.10) | 0 | 1 (0.07) |
| Congenital malformations of the nervous system Q00–Q07 | 1 (0.10) | 7 (0.05) | 0 | 0 |
| Congenital malformations of eye, ear, face and neck Q10–Q18 | 1 (0.10) | 13 (0.09) | 0 | 1 (0.07) |
| Congenital malformations of the circulatory system Q20–Q28 | 3 (0.31) | 126 (0.91) | 5 (0.73) | 9 (0.62) |
| Congenital malformations of the respiratory system Q30–Q34 | 2 (0.21) | 23 (0.17) | 1 (0.15) | 3 (0.21) |
| Cleft lip and cleft palate Q35–Q37 | 0 | 8 (0.06) | 0 | 1 (0.07) |
| Congenital malformations of the digestive system Q38–Q45 | 1 (0.10) | 19 (0.14) | 1 (0.15) | 1 (0.07) |
| Congenital malformations of genital organs Q50–Q56 | 1 (0.10) | 9 (0.06) | 0 | 0 |
| Congenital malformations of the urinary system Q60–Q64 | 1 (0.10) | 7 (0.05) | 1 (0.15) | 0 |
| Congenital malformations of the musculoskeletal system Q65–Q79 | 1 (0.10) | 25 (0.18) | 2 (0.29) | 2 (0.14) |
| Chromosomal abnormalities, not elsewhere classified Q90–Q99 | 0 | 5 (0.04) | 1 (0.15) | 0 |
| Hematologic abnormalities D50–D89 | 1 (0.10) | 7 (0.05) | 0 | 0 |
| Metabolic abnormalities E00–E90 | 0 | 6 (0.04) | 0 | 0 |
| Other congenital malformations Q80–Q89 | 0 | 3 (0.02) | 0 | 0 |

group, 0.82% in the vitrified day 5 blastocyst transfers group and 0.68% in the vitrified day 6 blastocyst transfers group. The corresponding proportion of any birth defect in twins was 1.70%, 2.86%, 3.54% and 2.52%, respectively.

Logistical analyses exploring the effect of embryo developmental stage at transfer on birth defects, adjusting for maternal age, infertility type, cause of infertility, year of birth, type of cycle (IVF/ICSI) and number of embryos transferred, did not find differences in the risk of birth defects between vitrified day 5 blastocyst transfers and vitrified day 3 embryo transfers among all births or in only singletons or twins (Table 4). Similarly, no differences in the risk of birth defects were found between vitrified day 6 blastocyst transfers and vitrified day 3 embryo transfers or between vitrified day 3 embryo transfers and fresh day 3 embryo transfers among all births or in only singletons or twins. Similar results were found when we studied specific organ systems.

## Discussion

Concerns regarding birth defects among infants born following ART have been raised since the first child conceived via IVF was born in 1978. Over the past decades, many studies were conducted to explore factors affecting the risk of birth defects after ART. Davies et al. reported that maternal factors, including maternal age and nulliparity, were associated with the risk of birth defects after ART [8]. Boulet et al. found that the infertility etiology and number of embryos transferred could affect the risk of birth defects [9]. The risk of specific types of birth defects was different between IVF and ICSI [10]. In the present study, the factors such as maternal age, infertility type, causes of infertility, type of cycle (IVF/ICSI) and number of embryos transfer were distributed differently between children born after vitrified blastocyst transfers and those born after fresh and vitrified cleavage-stage embryo transfers, which may also affect the risk of birth defects. So we adjusted these factors in estimating the effect of embryo developmental stage at transfer on birth defects.

In the present study, 1.10–1.75% of ART-conceived infants had at least one major birth defects, which was consistent with two previous studies conducted in China. A multicenter study conducted among 15,405 Chinese offspring born after ART in 7 reproductive medical centers reported the incidence rate of birth defects was between 1.11 and 1.58% [11]. A retrospctive study conducted in an assisted reproduction center of Shaanxi province found 7 births with birth defects among 494 babies delivered from vitrified embryo transfers [12]. However, the study performed in Belgium reported a higher incidence of birth defects than our study, in which 2.6% of singletons and 2.4% of twins following vitrified embryo transfer had birth defects [13]. A retrospective single-centre cohort study in Japan reported that the birth defects' rates were 2.4% for singletons born after vitrified embryo transfer and 1.9% for fresh embryo transfer [14].

The main finding of this large retrospective cohort study was that the risk of birth defects was not associated with embryo developmental stage at transfer among all births or in only singletons or twins. More specifically, there is

**Table 3** Proportion for birth defects of singleton and twins infants conceived with cleavage-stage or blastocyst-stage transfers in China, 2005–2017

| | Fresh day 3 embryo transfers | Vitrified day 3 embryo transfers | Vitrified day 5 blastocyst transfers | Vitrified day 6 blastocyst transfers |
|---|---|---|---|---|
| Singleton | 719 | 10,242 | 489 | 1175 |
| Any defect | 7 (0.97) | 138 (1.35) | 4 (0.82) | 8 (0.68) |
| Multiple defects | 1 (0.14) | 7 (0.07) | 0 | 0 |
| Congenital malformations of the nervous system Q00–Q07 | 1 (0.14) | 2 (0.02) | 0 | 0 |
| Congenital malformations of eye, ear, face and neck Q10–Q18 | 1 (0.14) | 9 (0.09) | 0 | 0 |
| Congenital malformations of the circulatory system Q20–Q28 | 1 (0.14) | 66 (0.64) | 1 (0.20) | 4 (0.34) |
| Congenital malformations of the respiratory system Q30–Q34 | 2 (0.28) | 12 (0.12) | 0 | 1 (0.09) |
| Cleft lip and cleft palate Q35–Q37 | 0 | 7 (0.07) | 0 | 1 (0.09) |
| Congenital malformations of the digestive system Q38–Q45 | 1 (0.14) | 10 (0.10) | 0 | 1 (0.09) |
| Congenital malformations of genital organs Q50–Q56 | 0 | 5 (0.05) | 0 | 0 |
| Congenital malformations of the urinary system Q60–Q64 | 1 (0.14) | 5 (0.05) | 1 (0.20) | 0 |
| Congenital malformations of the musculoskeletal system Q65–Q79 | 1 (0.14) | 18 (0.18) | 2 (0.41) | 1 (0.09) |
| Chromosomal abnormalities, not elsewhere classified Q90–Q99 | 0 | 3 (0.03) | 0 | 0 |
| Hematologic abnormalities D50–D89 | 0 | 3 (0.03) | 0 | 0 |
| Metabolic abnormalities E00–E90 | 0 | 3 (0.03) | 0 | 0 |
| Other congenital malformations Q80–Q89 | 0 | 3 (0.03) | 0 | 0 |
| Twins | 235 | 3675 | 198 | 278 |
| Any defect | 4 (1.70) | 105 (2.86) | 7 (3.54) | 7 (2.52) |
| Multiple defects | 0 | 7 (0.19) | 0 | 1(0.36) |
| Congenital malformations of the nervous system Q00–Q07 | 0 | 5 (0.14) | 0 | 0 |
| Congenital malformations of eye, ear, face and neck Q10–Q18 | 0 | 4 (0.11) | 0 | 0 |
| Congenital malformations of the circulatory system Q20–Q28 | 2 (0.85) | 60 (1.63) | 4 (2.02) | 5 (1.80) |
| Congenital malformations of the respiratory system Q30–Q34 | 0 | 11 (0.30) | 1 (0.51) | 2 (0.72) |
| Cleft lip and cleft palate Q35–Q37 | 0 | 1 (0.03) | 0 | 0 |
| Congenital malformations of the digestive system Q38–Q45 | 0 | 9 (0.24) | 1 (0.51) | 0 |
| Congenital malformations of genital organs Q50–Q56 | 1 (0.43) | 4 (0.11) | 0 | 0 |
| Congenital malformations of the urinary system Q60–Q64 | 0 | 2 (0.05) | 0 | 0 |
| Congenital malformations of the musculoskeletal system Q65–Q79 | 0 | 7 (0.19) | 0 | 1 (0.36) |
| Chromosomal abnormalities, not elsewhere classified Q90–Q99 | 0 | 2 (0.05) | 1 (0.51) | 0 |
| Hematologic abnormalities D50–D89 | 1 (0.43) | 4 (0.11) | 0 | 0 |
| Metabolic abnormalities E00–E90 | 0 | 3 (0.08) | 0 | 0 |
| Other congenital malformations Q80–Q89 | 0 | 0 | 0 | 0 |

no difference in the risk of birth defects between vitrified blastocyst-stage transfers and vitrified cleavage-stage transfers (including day 5 vs. day 3 and day 6 vs. day 3) among all births or in only singletons or twins. For infants born after cleavage-stage embryo transfers at day 3, there is no difference in the risk of birth defects between fresh embryo transfers and vitrified embryo transfers among all births or in only singletons or twins.

The practice of extended embryo culture to the blastocyst stage has been gradually increasing over the past decades. The blastocyst stage is thought to be a physiologically more appropriate time for transfer because it more closely mimics the natural implantation time and could improve synchronicity between the endometrium and embryo development [15, 16]. Previous studies have found an increased pregnancy rate and live birth rate after blastocyst transfer compared with cleavage-stage transfer [17–19]. However, there are some drawbacks with blastocyst culture [20]. One drawback is the possibility of transfer cancellation because some embryos could not reach the blastocyst stage. In addition, studies in animals have found altered expression of genes related to apoptosis, oxidative stress and gap junction formation in extended culture embryos [2, 21]. Moreover, murine studies have found that the practice of prolonged culture to the blastocyst stage could affect embryonic epigenetic reprogramming and modify epigenetic markers [22]. The results describing the effect of extended culture on birth defects are inconsistent. In the present study, the finding of

**Table 4** Risk of birth defects among infants conceived with cleavage-stage or blastocyst-stage transfers in China, 2005–2017

| | All births aOR(95% CI)[a] | Singleton births aOR(95% CI)[a] | Twin births aOR(95% CI)[a] |
|---|---|---|---|
| Any birth defects | | | |
| Vitrified embryo transfer | | | |
| Blastocyst-stage transfer day 5 vs. cleavage-stage transfer Day 3 | 1.34 (0.70,2.57) | 0.68 (0.24,1.97) | 1.93 (0.86,4.35) |
| Blastocyst-stage transfer day 6 vs. cleavage-stage transfer day 3 | 0.73 (0.43,1.25) | 0.54 (0.25,1.16) | 0.88 (0.40,1.95) |
| Cleavage-stage transfer day 3 | | | |
| Vitrified embryo transfer vs. fresh embryo transfer | 0.72 (0.36,1.44) | 0.66 (0.28,1.58) | 0.77 (0.23,2.60) |
| Congenital malformations of the circulatory system | | | |
| Vitrified embryo transfer | | | |
| Blastocyst-stage transfer day 5 vs. cleavage-stage transfer day 3 | 1.46 (0.57,3.75) | 0.50 (0.06,3.87) | 1.95 (0.67,5.65) |
| Blastocyst-stage transfer day 6 vs. Cleavage-stage transfer day 3 | 0.88 (0.43,1.78) | 0.67 (0.23,1.93) | 1.08 (0.41,2.81) |
| Cleavage-stage transfer Day 3 | | | |
| Vitrified embryo transfer vs. fresh embryo transfer | 1.17 (0.34,4.02) | 1.63 (0.22,11.98) | 0.81 (0.15,4.33) |
| Congenital malformations of the respiratory system[b] | | | |
| Vitrified embryo transfer | | | |
| Blastocyst-stage transfer day 5 vs. cleavage-stage transfer day 3 | 0.95 (0.11,8.20) | | |
| Blastocyst-stage transfer day 6 vs. cleavage-stage transfer day 3 | 1.06 (0.28,4.02) | | |
| Cleavage-stage transfer day 3 | | | |
| Vitrified embryo transfer vs. fresh embryo transfer | 0.24 (0.05,1.06) | | |
| Congenital malformations of the digestive system[b] | | | |
| Vitrified embryo transfer | | | |
| Blastocyst-stage transfer day 5 vs. cleavage-stage transfer day 3 | 1.89 (0.22,16.41) | | |
| Blastocyst-stage transfer day 6 vs. cleavage-stage transfer day 3 | 0.48 (0.06,3.95) | | |
| Cleavage-stage transfer day 3 | | | |
| Vitrified embryo transfer vs. fresh embryo transfer | 0.86 (0.06,12.40) | | |
| Congenital malformations of the musculoskeletal system[b] | | | |
| Vitrified embryo transfer | | | |
| Blastocyst-stage transfer day 5 vs. cleavage-stage transfer day 3 | 1.61 (0.33,7.85) | 2.45 (0.44,13.61) | |
| Blastocyst-stage transfer day 6 vs. cleavage-stage transfer day 3 | 0.98 (0.22,4.34) | 0.66 (0.08,5.36) | |
| Cleavage-stage transfer day 3 | | | |
| Vitrified embryo transfer vs. Fresh embryo transfer | 0.52 (0.07,3.97) | 0.36 (0.05,2.85) | |

[a]Adjusted by maternal age, infertility type, causes of infertility, year of birth, type of cycle (IVF/ICSI) and number of embryos transfer

[b]OR was not calculated because of the few cases

no change in the risk of birth defects between blastocyst-stage transfers and cleavage-stage transfers concurs with a previous study performed in Canada, although that study only included singleton births from fresh embryo transfer cycles [6]. However, a Swedish study reported an increase in birth defects after blastocyst-stage transfers compared with cleavage-stage transfers among 13,873 infants from both fresh and frozen embryos [5]. Randomized, controlled trials and follow-up studies of the long-term outcomes of children born after blastocyst-stage transfers are needed to confirm the clinical safety of extending embryo culture to the blastocyst stage.

Embryo cryopreservation has been a routine part of clinical IVF practices, as it helps to optimize the clinical use of excess embryos and improve the cumulative live birth rate per oocyte retrieval cycle. Although cryopreservation has been widely used in recent years, there are still concerns about its clinical safety. To date, most published reports have concentrated on children born after vitrified blastocyst-stage embryo transfers [23–25]. A few existing studies comparing neonatal outcomes after vitrified day 3 embryo transfers with fresh day 3 embryo transfers have suggested that the cryopreservation of day 3 cleavage-stage embryos is a safe method [12, 26]. These researchers speculated that it involves a process of self-selection, such that only good quality embryos could survive the freezing and thawing process and develop into blastocysts. However, most of these studies focused on neonatal outcomes, such as gestational age and birth weight, and few studies examined the effect of frozen embryo transfer on birth defects. Although Rama

Raju reported the birth defect rate in his study, the small sample size decreased the power of the study. In addition, some authors have suggested that embryo cryopreservation may cause modifications of the genome and affect the development of the fetus, highlighting the importance of exploring the effect of cryopreservation on birth defects [27]. In the present study, we evaluated the risk of birth defects after vitrified day 3 embryo transfers compared with fresh day 3 embryo transfers. Although the proportion of any birth defects after vitrified day 3 embryo transfers was higher than that after fresh day 3 embryo transfers among all births (1.75% vs. 1.15%) and only singletons (1.35% vs. 0.97%) or twins (2.86% vs. 1.70%), these differences were not significant after adjusting for potential confounding factors.

This was a large retrospective cohort study. We compared the risk of birth defects among singleton and twin infants born after vitrified blastocyst transfers with those after vitrified cleavage embryo transfers (including day 5 vs. day 3 and day 6 vs. day 3). In addition, we compared the risk of birth defects for infants born after vitrified day 3 embryo transfers with those born after fresh day 3 embryo transfers. However, this study also had some limitations. First, because of the limited number of fresh blastocysts transferred, we could not compare fresh blastocyst transfer with fresh cleavage embryo transfer or vitrified blastocyst transfer. Second, we had no information about the occurrence of birth defects among women who suffered miscarriage due to fetal abnormality, which may result in the underestimation of the true prevalence of birth defects [28, 29]. Third, we were unable to adjust for some potentially confounding variables, such as prenatal screening for fetal abnormalities, family history of birth defects, maternal smoking, parental smoking and environmental exposures [30–32].

Prolonged culture to the blastocyst stage has been the preferred strategy in recent years. The main advantage of this strategy is the higher live birth rate per embryo transfer cycle. However, for clinicians, healthcare providers or public health professionals, the ultimate goal of ART is to achieve a healthy live birth. Thus, we should consider other short- and long-term health outcomes of children born after blastocyst-stage transfer in addition to the live birth rate before making the decision to recommend a blastocyst transfer in clinical practice.

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**Author contributions** QQZ: Manuscript writing, data analysis; NLW: Data analysis; BW: Data collection; YW: Data collection; YPK: Project development.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** All participants gave written informed consent for this research.

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