The effects of number and quality of transferred blastocysts and female age on pregnancy outcomes in frozen-thawed cycles: a retrospective study

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Abstract

Abstract Objective To evaluate the effects of number and quality of transferred blastocysts and female age on pregnancy outcomes. Design A retrospective study. Setting Two IVF centers. Population Women undergoing first blastocyst transfer in frozen-thawed cycles. Methods 6096 frozen-thawed blastocyst transfer cycles were divided into five groups based on blastocyst number and quality and three groups according to female age. Main Outcome Measures Implantation rate (IR), clinical pregnancy rate (CPR), multiple pregnancy rate (MPR), live birth rate (LBR) and neonatal characteristics. Results Female age, quality and number of transferred blastocysts had significant effects on pregnancy outcomes. IR, CPR and LBR of the same blastocyst transfer groups decreased gradually with age. Increasing the transferred number of same grade blastocysts did not obviously increase CPR and LBR, however, it significantly increased MPR. For double blastocyst transfer, the better-quality the transferred blastocysts were, the higher the IR, CPR, MPR and LBR were. Singleton group had the best neonatal characteristics followed by dizygotic twins and monozygotic twins. There was a positive correlation between sex ratio and the proportion of good-quality blastocysts, and the difference was significant between good-quality and poor-quality blastocyst groups (1.35 vs 0.98, P/CI = 0.002/1.123-1.687). Conclusions Selective single good-quality blastocyst transfer may serve as an effective embryo transfer strategy to reduce MPR while maintaining LBR and obtain good pregnancy outcomes, but, the strategy may result in an unbalanced sex ratio of newborns. Funding None. Keywords: Blastocyst transfer, age, multiple pregnancy, live birth rate, neonatal characteristics.

Introduction

With the development of assisted reproductive technologies, particularly in embryo culture and cryopreservation, the clinical pregnancy rate (CPR) is increasing along with the multiple pregnancy rate (MPR). Multiple pregnancy is considered the most significant adverse event associated with ART and is linked to an increased risk of maternal and neonatal morbidity. Reducing the number of transferred embryos is a key factor in reducing multiple pregnancies.[1] However, this method has not been widely used in clinical practice due to concerns pertaining to the lower pregnancy rate following the reduction of the number of transferred embryos. Compared to cleavage-stage embryo, extending embryo culture to blastocyst allows for better evaluation of the implantation potential of embryo, and the implantation rate (IR) of blastocyst is higher.[2-4]Therefore, selective single top-quality blastocyst transfer should serve as a practical strategy in reducing the number of transferred embryos while maintaining CPR. Previous findings have suggested that comparable CPR could be achieved in a clinical setting when utilizing elective single blastocyst transfer in regard to patients with a good prognosis. 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] Moreover, frozen single blastocyst transfer resulted in a higher CPR compared to that of fresh single blastocyst transfer in ovulatory women with a good prognosis.[6] In advanced female age ([?] 40 years), single blastocyst transfer and double blastocyst transfer achieved similar CPR and LBR, while MPR was lower in the single blastocyst group.[7] Although research on blastocyst transfer has been on the rise recently, these studies did not comprehensively analyze the effects of number and quality of transferred blastocysts as well as female age on pregnancy and clinical outcomes. In the present study, the effects of number and quality of transferred blastocysts and female age on clinical outcomes are compared in order to determine a more appropriate blastocyst transfer policy to decrease MPR while maintaining LBR.

Methods

Participants

This retrospective study was carried out from January 2016 to December 2019 at the Center for Reproductive Medicine of Mali Hospital, Haikou, China and the Hospital of Shenzhen Immigration Inspection, Shenzhen, China. This study included the first frozen-thawed blastocyst transfer cycle of woman who underwent IVF cycle with or without ICSI (Intracytoplasmic Sperm Injection) with an indication of tubal, male, or unexplained infertility. Women with a diagnosis of a congenital or acquired uterine abnormality, such as a uterine malformation, adenomyosis, submucous myoma, uterine fibroids or intrauterine adhesions were excluded from this study.

Study design

The 6096 frozen-thawed blastocyst transfer cycles were divided into five groups based on number and quality: a good-quality blastocyst (G), two good-quality blastocysts (GG), a good-quality blastocyst and a poor-quality blastocyst (GP), a poor-quality blastocyst (P), and two poor-quality blastocysts (PP). The patients were subsequently divided into three groups according to female age: < 35, 35-39 and > 39 years. Pregnancy outcomes included implantation rate (IR), clinical pregnancy rate (CPR), multiple pregnancy rate (MPR), abortion rate (AR), live birth rate (LBR), gestational age, mode of delivery, sex ratio, congenital malformation and birthweight. IR was defined as the number of gestational sacs on ultrasound over the total number of transferred blastocysts. Clinical pregnancy was defined as a gestational sac seen by vaginal ultrasound scan by 6-7 weeks of gestation, while multiple pregnancy was defined as having more than one gestational sac. Meanwhile, abortion was defined as the spontaneous cessation of a clinical pregnancy, and live birth was defined as delivery of a live fetus at gestational age [?] 24 weeks.

Embryo culture, freezing and thawing protocols

Embryo culture was carried out in Quinn's IVF sequential medium suite (Quinn's, SAGE, USA) after adding 10% human serum substitute (Quinn's, SAGE, USA) in 5% O_2, 5% CO_2, 90% N_2 and saturated humidity. Embryos were cultured individually in 25 µl microdroplet of cleavage medium covered with oil and then transferred from the cleavage medium to a blastocyst medium covered with oil on day 3. About 3 embryos were 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 or 6.[8]Good-quality blastocysts were given a numerical score from 3 to 6 according to their degree of expansion and hatching status, and the inner cell mass and trophectoderm were assessed as A or B, while other types of blastocysts were poor-quality.

Available blastocysts were vitrified using vitrification media (Kitazato Biopharma, Shizuoka, Japan). Briefly, blastocysts were transferred from the culture medium to the center of the equilibration solution and remained in the media for about 9 minutes at room temperature. Then, blastocysts were transferred into vitrification solution and blown and inhaled at different positions. After 40-50 s in this solution, the embryos were placed on the Cryotop (Kitazato Biopharma, Shizuoka, Japan) and rapidly placed into liquid nitrogen.

Blastocysts were thawed using vitrification media (Kitazato Biopharma, Shizuoka, Japan). Briefly, Cryotop was transferred rapidly from liquid nitrogen and immersed in a 300 µl droplet of thawing solution (TS) at 37. After 1 minute, the blastocysts were transferred to a 300 µl droplet of diluent solution (DS) at room temperature for 3 minutes. Then, the blastocysts were transferred to 300 µl droplets of washing solution 1 (WS1) and washing solution 2 (WS2) at room temperature for 5 minutes, respectively. Finally, the blastocysts were transferred to a 30 µl droplet of blastocyst medium and regarded before transfer.

Endometrial preparation

Natural cycle and artificial cycle were used for frozen-thawed blastocyst transfer (FET). Natural cycle was conducted either by administration of HCG for planning the transfer or by detecting the spontaneous LH peak, and blastocysts were transferred on the fifth day after ovulation. In terms of the artificial cycle, 3.75 mg per day of oral oestradiol valerate was started on days 2-3 of the menstrual cycle, and the dose of estradiol valerate was adjusted according to the endometrial thickness monitored by ultrasound. When the endometrial thickness reached [?] 7 mm, 40 mg/d progesterone was injected 5 days prior to frozen blastocyst transfer. If pregnancy was achieved after frozen blastocyst transfer, luteal phase support was continued until 11 weeks' gestation.

Statistical analyses

Statistical analyses were performed using SPSS 22.0 (IBM, Armonk, USA). Quantitative variables were presented as means $\pm$ standard deviations and categorical variables were expressed as frequencies and percentages. The data were analyzed using the Chi-squared test, Fisher's exact test, one-way analysis of variance (ANOVA) or Bonferroni testas appropriate. Logistic regression analysis was performed to explore the effects of different blastocyst transfer strategies on live birth after controlling for potential confounders including female age, female BMI, duration of infertility and endometrial thickness. A two-sided $P < 0.05$ was considered to be statistically significant.

Results

Patient and cycle characteristics

This study included 6096 frozen-thawed blastocyst transfer cycles. The female 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 are shown in Table S1, where no significant differences were observed among the < 35, 35-39 and > 39 age groups. The differences of body-mass index, natural cycle rate and duration of infertility were found to be significant in the total age group (P values were all less than 0.01).

Pregnancy outcomes

Table 1 presents the effects of quality and number of transferred blastocysts on pregnancy outcomes. The results indicated that the quality and number of transferred blastocysts had significant effects on pregnancy outcomes. In the total age group, IR (75.3%) and LBR (62.8%) were observed to be highest in group G, while CPR (77.2%) and MPR (56.5%) were the highest in group GG. Additionally, AR was relatively high in groups P (17.2%) and PP (16.9%). Compared to groups without good-quality blastocysts (groups P and PP), CPR and LBR were higher and AR was lower in groups with good-quality blastocysts (groups G, GG and GP). In the groups with good-quality blastocysts, Group G had higher IR (P /OR/CI = 0.00/2.33/1.99-2.74 and 0.00/2.91/2.57-3.29) and lower MPR (P /OR/CI = 0.00/0.02/0.02-0.03 and 0.00/0.05/0.04-0.06) than groups GG and GP, and their CPR, AR and LBR were similar. In the groups without good-quality blastocysts, group P also had higher IR (P /OR/CI = 0.00/1.80/1.43-2.25) and lower MPR (P /OR/CI = 0.00/0.02/0.01-0.08) than group PP, and their CPR, AR and LBR possessed no significant differences. These results indicated that increasing the number of transferred blastocysts classified as same grade does not obviously increase CPR and LBR, but it would significantly increase MPR. For double blastocyst transfer (groups GG, GP and PP), the better the quality of blastocysts transferred, the higher the IR, CPR, MPR and LBR would be, and these rates were found to decrease significantly in group PP (GG vs PP, P /OR/CI = 0.00/2.07/1.74-2.47, 0.00/2.22/1.68-2.94, 0.00/2.62/1.94-3.55 and 0.00/2.02/1.56-2.62 respectively). For single blastocyst transfer, group G had a higher IR, CPR and LBR (P /OR/CI = 0.00/2.69/2.17-3.33, 0.00/2.69/2.17-3.33, 0.00/2.70-/2.06-3.54 respectively) and a low AR (P /OR/CI = 0.17/0.76/0.52-1.11) compared to group P.

In different age groups, the results indicated that IR, CPR and LBR of same blastocyst transfer groups decreased gradually with age, and these rates decreased significantly when female age was more than 39 years old (P values were all less than 0.05). MPR of groups GG, GP and PP were found to decrease gradually with age (P = 0.00, 0.00 and 0.00 respectively). Compared to group < 35, AR was found to be significantly increased in group > 39 (P values were all less than 0.05). In each age group, the changing trends of IR, CPR, MPR, AR and LBR were similar to that in total age group.

A logistic regression model for predicting live birth is given in Table 2. Variables that were statistically significant in Table S1, or those that were deemed to be clinically significant, were included in order to build the model. This model indicated that the most significant predictors for live birth were female age, type of infertility and transferred blastocysts. LBR of the same blastocyst transfer strategies groups decreased gradually with age, and groups with good-quality blastocysts (groups G, GG and GP) had a higher LBR than those without good-quality blastocysts (groups PP and P) (P /OR/CI = 0.00/2.10/1.81-2.43).

Neonatal characteristics

Tables 3 and 4 present the neonatal characteristics of live born singletons and twins in the total blastocyst transfer group and different blastocyst transfer groups. The results indicated that the singleton group had a higher average gestational age (P /OR/CI = 0.00/2.71/2.47-2.94) and birthweight (P /MD/CI = 0.00/826.62/778.92-874.32) as well as a lower cesarean section rate (P /OR/CI = 0.00/0.09/0.06-0.14), preterm labor rate (P /OR/CI = 0.00/0.06/0.04-0.07) and low birthweight rate (P /OR/CI = 0.00/0.05/0.04-0.07) than the twin group. Moreover, no significant differences were present in regard to sex ratio and congenital malformation rate between the two groups (P /OR/CI = 0.67/1.04/0.88-1.23 and 0.47/0.79/0.42-1.49). Table 4 illustrates that blastocyst transfer strategies had significant effects on gestational age, cesarean section and birthweight in the singleton group (P =0.00, 0.00 and 0.01 respectively). Monozygotic twins had a lower gestational age (34.9 ± 3.0 vs 35.9 ± 2.1, P /MD/CI = 0.01/-0.99/-1.77–0.20) and birthweight (2228.8 ± 502.8 vs 2485.6 ± 406.2, P /MD/CI = 0.00/-256.8/-406.6–107.1) as well as a higher preterm birth rate (75.8% vs 57.1%, P /OR/CI =0.01/3.12/1.25-7.83) and low birthweight rate (66.7% vs 44.3%,P /OR/CI = 0.00/2.52/1.48-4.30) compared to dizygotic twins. The sex ratio of good-quality embryo groups (groups G and GG) (1.35, 1349/998) was highest, followed by the GP group (1.16, 321/276) and poor-quality embryo groups (groups P and PP) (0.98, 219/223). Additionally, the difference was significant between good-quality and poor-quality embryo groups (P /OR/CI = 0.00/1.38/1.12-1.69).

Discussion

Main Findings

In this retrospective study, the results indicated that female age, quality and number of transferred blastocysts had significant effects on pregnancy outcomes. IR, CPR and LBR of the same blastocyst transfer groups were observed to decrease gradually with age. Moreover, increasing the transferred number of same grade blastocysts did not obviously increase CPR and LBR, but, it significantly increased MPR. For double blastocyst transfer, the better-quality the transferred blastocysts were, the higher the IR, CPR, MPR and LBR were. In addition, the singleton group had a higher average gestational age and birthweight as well as a lower cesarean section rate, preterm labor rate and low birthweight rate than the twin group. Monozygotic twins had a lower gestational age and birthweight in conjunction with a higher preterm birth rate and low birthweight rate than dizygotic twins. There was a positive correlation between sex ratio (male/female) and the proportion of good-quality blastocysts, and the difference was found to be significant between good-quality and poor-quality blastocyst groups.

Strengths and Limitations

The study had some advantages in its design that afforded strong confidence on the results. Firstly, the study was based on a large sample including 6096 FET cycles. Then, the study divided the female age into three groups to decrease the effects of female age on clinical outcomes. Moreover, the study selected first FET cycle and excluded FET cycles with abnormal uterine to ensure the accuracy of data. One limitation of this study was that the blastocyst quality was not graded finely and only divided into two levels, which may have resulted in a difference of blastocyst quality in the same level and affect the pregnancy outcomes of different groups.

Interpretation

The clinical results of the total patient population indicated that group G had the highest IR, lowest MPR and similar CPR, AR and LBR among groups with good-quality blastocysts, which were similar to those of previous studies.[9-11]Moreover, group P also had a higher IR, lower MPR, and similar CPR, AR and LBR between groups without good-quality blastocysts, which were also in accord with those of previous studies.[12] In regard to double blastocyst transfer, the MPR of groups GG, GP and PP were 56.5%, 36.3% and 33.1%, respectively. The changed trends of pregnancy results for different age groups were observed to be equivalent with those for the total patient population. Accordingly, the obtained results indicated that single blastocyst transfer was an effective embryo transfer strategy in significantly reducing the MPR while maintaining LBR. The results of the logistic regression analysis demonstrated that female age and transferred blastocysts were significantly relative to LBR, and the MPR of groups GG, GP and PP were 67.6%, 41.0% and 40.9%, respectively, in $<$ 35 age group. Therefore, it is suggested that single blastocyst transfer should be recommended rather than any double blastocyst transfer methods, and transferring two good-quality blastocysts should be avoided in young patients. Furthermore, increasing the number of blastocysts transferred classified as the same grade was not found to obviously increase CPR and LBR, however, it significantly increased MPR. The corresponding results were similar to those of previous studies.[10, 13, 14]

Pregnancy is widely viewed as being dependent on an intimate dialogue mediated by locally secreted factors between a developmentally competent embryo and receptive endometrium. Previous studies have indicated that decidualizing human endometrial stromal cells could selectively recognize the presence of a developmentally impaired embryo and respond by inhibiting the secretion of key implantation mediators (e.g. IL-1β and HB-EGF) and immunomodulators (e.g. IL-5, -6, -10, -11, -17, and eotaxin).[15] Impaired human embryos elicited an endoplasmic stress response in human decidual cells, and signals emanating from developmentally competent embryos activated a focused gene network enriched in metabolic enzymes and implantation factors.[16] Moreover, competent human embryos triggered short-lived oscillatory Ca21 fluxes, whereas low-quality embryos caused a heightened and prolonged Ca21 response.[16] These distinct positive and negative mechanisms may result in a lower IR of poor-quality blastocysts. Additionally, its inhibiting effect may be a superimposed effect resulting in a lower IR of two poor-quality blastocysts than one poor-quality blastocyst. The IR of a single good-quality blastocyst was found to be higher than that of two good-quality blastocysts, which may be because the secretion of implantation factors was insufficient for implantation of the two blastocysts. The IR of GP was lower than that of P and G in groups $<$ 35, 35-39 and total patient population, which was in accord with previous studies.[10, 12] The reason for this result may be that biochemical reactions preventing the implantation of the poor-quality blastocyst have a negative influence on the implantation of the good-quality blastocyst. Additionally, a competitive relationship may exist between transferred blastocysts, which results in a lower IR of double blastocysts than one blastocyst. Previous findings have 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.[12] Blastocyst score and proportion of top-scoring blastocyst affected implantation, and the degree of blastocoele re-expansion served as a significant ability in predicting live birth in a warmed single blastocyst transfer cycle.[17, 18] These results were similar to our study.

Multiple pregnancy is considered the most significant adverse event associated with assisted reproductive technologies and linked to an increased risk of maternal and neonatal morbidity. In this study, the singleton group had a higher average gestational age and birthweight as well as a lower cesarean section rate, preterm labor rate and low birthweight rate than the twin group. Moreover, the LBR of single blastocyst transfer was similar to that of double blastocyst transfer in good-quality blastocyst transfer groups and poor-quality blastocyst transfer groups. Hence, single blastocyst transfer is recommended in decreasing the risk of maternal and neonatal morbidity caused by multiple pregnancy. Monozygotic twins had a lower gestational age and birthweight in conjunction with a higher preterm birth rate and low birthweight rate compared to dizygotic twins. Therefore, the monozygotic twin deserves more attention in assisted reproductive technologies.

In vitro culture induced precocious X-chromosome inactivation and ICSI induced decrease in number of trophectoderm cells in female blastocysts.[19] Sex ratio was significantly higher toward males in the transfer of blastocyst compared to transfers of cleavage stage embryos.[19-22] Our findings also indicated the presence of a positive correlation between sex ratio and blastocyst quality, which was significant between good-quality and poor-quality embryo groups. Additionally, blastocyst culture and selective single good-quality blastocyst transfer are being recommended by an increasing number of researchers.[6, 10, 23] Thus, this transfer strategy may result in an unbalanced sex ratio of newborns in the future, and further research is required in this area.

Conclusions

In conclusion, selective single good-quality blastocyst transfer was found to serve as an effective embryo transfer strategy in significantly reducing MPR while maintaining LBR. Any double blastocyst transfer methods are not recommended and the transfer strategy may result in an unbalanced sex ratio of newborns in the future. Moreover, monozygotic twin deserves more attention in assisted reproductive technologies.

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Disclosure of Interests

The authors declare that they have no conflict of interest.

Contribution to Authorship

The study was designed by YHL and BM. LGZ, XXC, NL and YHL were involved in planning and managing the data collection. YHL and BM were involved in the statistical analysis and wrote the manuscript with support and critical review from all authors.

Details of Ethical Approval

Ethical approval was granted by the Ethics Committee of Mali Hospital on the March 8, 2020 (NO. 02/MLYY/2020).

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References

1 Sullivan EA, Wang YA, Hayward I, Chambers GM, Illingworth P, McBain J, et al. Single embryo transfer reduces the risk of perinatal mortality, a population study.*Hum Reprod* 2012; 27: 3609-15.

2 Kaur P, Swarankar ML, Maheshwari M, Acharya V. A comparative study between cleavage stage embryo transfer at day 3 and blastocyst stage transfer at day 5 in in-vitro fertilization/intra-cytoplasmic sperm injection on clinical pregnancy rates. *J Hum Reprod Sci* 2014; 7: 194-7.

3 Fernandez SS, Cercas R, Brana C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results.*J Assist Reprod Genet* 2015; 32: 177-84.

4 Glujovsky D, Farquhar C. Cleavage-stage or blastocyst transfer: what are the benefits and harms?*Fertil Steril* 2016; 106: 244-50.

5 Jacobs MB, Klonoff CH, Garzo VG. Equivalency of In vitro fertilization success rates in elective single blastocyst transfer and elective double blastocyst transfer: an example of equivalence methodology in clinical reproductive health. *J Hum Reprod Sci* 2018; 11: 45-51.

6 Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ, et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial.*The Lancet* 2019; 393: 1310-8.

7 Tannus S, Son WY, Dahan MH. Elective single blastocyst transfer in advanced maternal age. *J Assist Reprod Genet* 2017; 34: 741-8.

8 Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer.*Fertil Steril* 2000; 73: 1155-8.

9 Wintner EM, Hershko KA, Tzadikevitch K, Ghetler Y, Gonen O, Wintner O, et al. Does the transfer of a poor quality embryo together with a good quality embryo affect the In Vitro Fertilization (IVF) outcome? *J Ovarian Res* 2017; 10: 1-5.

10 Park DS, Kim JW, Eum JH, Lee WS, Yoon TK, Lyu SW. Clinical and pregnancy outcomes of double and single blastocyst transfers related with morphological grades in vitrified-warmed embryo transfer. *Taiwan J Obstet Gynecol* 2020; 59: 398-402.

11 Li J, Du M, Zhang Z, Guan Y, Wang X, Zhang X, et al. Does a poor-quality embryo have an adverse impact on a good-quality embryo when transferred together? *J Ovarian Res* 2018; 11: 1-5.

12 EI DI, Sterzik K, Rinaldi L, Pacchiarotti A, De SM, Selman H. Effect of transferring a morphologically impaired embryo with a good quality embryo on the pregnancy and implantation rates. *Eur Rev Med Pharmacol Sci* 2016; 20: 394-8.

13 Mullin CM, Fino ME, Talebian S, Krey LC, Licciardi F, Grifo JA. Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age. *Fertil Steril* 2010; 93: 1837-43.

14 Zhu Q, Lin J, Gao H, Wang N, Wang B, Wang Y. The association between embryo quality, number of transferred embryos and live birth rate after vitrified cleavage-stage embryos and blastocyst transfer. *Front Physiol* 2020; 11: 1-7.

15 Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, et al. Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS One* 2010; 5: 1-9.

16 Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Lucas ES, et al. Uterine selection of human embryos at implantation. *Sci Rep* 2014; 4: 1-13.

17 Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome towards a single blastocyst transfer.*Fertil Steril* 2000; 73: 1155-8.

18 Du QY, Wang EY, Huang Y, Guo XY, Xiong YJ, Yu YP, et al. Blastocoele expansion degree predicts live birth after single blastocyst transfer for fresh and vitrified/warmed single blastocyst transfer cycles. *Fertil Steril*2016; 105: 910-9.

19 Tarín JJ, García-Pérez MA, Hermenegildo C, Cano A. Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment cycles. *Reprod Biol Endocrinol* 2014; 12: 1-13.

20 Bu Z, Chen ZJ, Huang G, Zhang H, Wu Q, Ma Y, et al. Live birth sex ratio after in vitro fertilization and embryo transfer in China–an analysis of 121,247 babies from 18 centers. *PLoS One* 2014; 9: 1-9.

21 Chen M, Du J, Zhao J, Lv H, Wang Y, Chen X, et al. The sex ratio of singleton and twin delivery offspring in assisted reproductive technology in China. *Sci Rep*2017; 7: 1-9.

22 Hentemann MA, Briskemyr S, Bertheussen K. Blastocyst transfer and gender: IVF versus ICSI.*J Assist Reprod Genet* 2009; 26: 433-6.

23 Freeman MR, Hinds MS, Howard KG, Howard JM, Hill GA. Guidance for elective single-embryo transfer should be applied to frozen embryo transfer cycles. *J Assist Reprod Genet* 2019; 36: 939-46.

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