Front. Med. 2017, 11(4): 563–569
https://doi.org/10.1007/s11684-017-0535-6

RESEARCH ARTICLE

Propensity score-matched study and meta-analysis of cumulative outcomes of day 2/3 versus day 5/6 embryo transfers

Ye Yin[1,*], Ge Chen[1,*], Kezhen Li[2], Qiuyue Liao[1], Sijia Zhang[1], Nieying Ma[1], Jing Chen[2], Yan Zhang[2], Jihui Ai (✉)[1]

[1]*Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China;* [2]*Department of Obstetrics and Gynecology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

Abstract **The superiority of the cumulative outcomes of day 5/6 embryo transfer to those of day 2/3 embryo transfer in infertile couples has been debated. This retrospective study included data collected from 1051 patients from July 2011 to June 2014. Multiple maternal baseline covariates were subjected to propensity score matching analysis, and each day 5/6 group woman was matched to one day 2/3 group woman. A systematic meta-analysis was conducted to validate the results. After matching was completed, 217 patients on the day 2/3 group were matched with those on the day 5/6 group, and no significant differences in the baseline characteristics were observed between the two groups. The cumulative pregnancy rate (57.14% vs. 53.46%, OR 1.16, 95% CI 0.79–1.70) and cumulative live birth rate (53.00% vs. 49.77%, OR 1.14, 95% CI 0.78–1.66) of day 5/6 embryo transfers were higher than those of day 2/3 embryo transfers, but this difference was not significant. The mean cycles per live birth and mean days per live birth in the day 5/6 group were significantly lower than those in the day 2/3 group. This study demonstrated that day 5/6 embryo transfer is a more cost-effective and time-efficient policy than day 2/3 embryo transfer to produce a live baby.**

Keywords blastocyst; embryo transfer; cumulative pregnancy rate; cumulative live birth rate; IVF

Introduction

Extending culture embryos until day 5/6 for transfer has emerged as a widely used IVF procedure worldwide. Compared with the transfer of day 2/3 embryos, day 5/6 embryo transfer provides several theoretical advantages, including high rate of implantation, selection of viable embryos for transfer, and potential reduction of the ectopic pregnancy rate [1–5]. However, day 5/6 embryo transfer is limited by the failure of most embryos to reach day 5/6 because of the complexity of *in vivo* environments [6,7]. As such, clinicians and patients consider the possibility of no embryo or low-viability embryo production for transfer if they choose day 5/6 embryo transfer. Studies have also yet to determine whether day 5/6 embryo transfer is superior to day 2/3 embryo transfer.

Pregnancy outcomes have been compared between day 5/6 and day 2/3 embryo transfers [8]. However, freeze–thaw cycles have been disregarded. Embryo cryopreservation following IVF cycle completion leads to a high probability of successful embryo survival. Freeze–thaw cycles provide a more physiological environment, and this advantage can enhance the pregnancy rates and reduce maternal and perinatal morbidity [9]. The cumulative outcome completion of fresh and frozen embryo transfers per oocyte retrieval cycles should be considered to measure IVF clinical outcomes appropriately [10,11].

With the progression of cryopreservation technology, vitrification has been considered the preferred method over traditional slow freezing methods [12,13]. Vitrification is associated with a significantly increased post-thaw survival rate, a high clinical pregnancy rate, and an on-going pregnancy rate in reviews and meta-analyses [14,15].

To our knowledge, few studies have been conducted on cumulative outcomes in patients with day 2/3 or day 5/6 embryo transfer [16]. These studies have also been limited by sample size, low freeze–thaw rate, and traditional slow freezing protocol and thus have yielded relatively low survival rates in the day 5/6 group. Interrelated studies

Received October 31, 2016; accepted March 10, 2017

Correspondence: jihuiai@tjh.tjmu.edu.cn

*Ye Yin and Ge Chen contributed equally to this article.

have yet to evaluate the timing and economic benefits of day 2/3 or day 5/6 embryo transfer. Therefore, the benefits of day 5/6 embryo transfer remain controversial.

In summary, our study aimed to compare day 2/3 embryo transfer with day 5/6 embryo transfer in terms of cumulative outcomes (cumulative pregnancy rate, cumulative live birth rate), mean cycles per live birth, and mean days per live birth by using a verification method.

Materials and methods

Study design

This retrospective cohort analysis was conducted at the Reproductive Medicine Centre, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China. The study included consecutive couples that were scheduled for oocyte retrieval between July 2011 and June 2014. Other inclusion criteria were as follows: the number of cleavage stage embryos between 3 and 20 based on actual conditions; presence of normal uterine cavity; and absence of any contraindications to pregnancy. Exclusion criteria were as follows: mixed day 2/3 and day 5/6 transfer, surplus embryos with failed pregnancy until April 2016, vitrified oocyte cycles, donor oocyte cycles, PGD, spontaneous conception, and recurrent spontaneous abortion. All included couples were divided into two groups based on the embryo transfer methods. The individuals in the day 2/3 group received cleavage stage embryo transfer. In the day 5/6 group, all of the embryos were cultured to the blastocyst stage and then transferred. This study conformed to the *Declaration of Helsinki* for medical research involving human subjects, and approval was obtained from the Ethical Committee of the Tongji Medical College.

IVF

Sperm preparation, IVF, and embryo culture were performed in accordance with previously described methods [17,18]. Ovarian stimulation was conducted by using follicle-stimulating hormone (FSH) with gonadotrophin-releasing hormone (GnRH) agonists according to either a long or short protocol. Women were administered with human chorionic gonadotrophin (HCG) when the two leading follicles reached a mean diameter of 18 mm. Oocytes were retrieved 36–37 h after HCG was administered. IVF or ICSI was performed as appropriate, and semen quality was considered. The day of oocyte retrieval was set as day 0.

Embryo culture and embryo grading

Oocytes were incubated in G-IVF medium (Vitrolife) and were fertilized 3–4 h after retrieval. Normal fertilization was defined as zygotes with two pronuclei (2PN). Then, the fertilized oocytes were continuously cultured in G1 medium for 2 additional days. Morphological evaluation of the embryos was performed on day 2/3 based on the number of blastomeres, rate of fragmentation, multinucleation of the blastomeres, and early compaction [19]. Day 3 embryos were graded as I–IV. I–III grade embryos were defined as viable embryos and were transferred or frozen. Blastocyst quality was assessed according to Gardner's scoring system based on the expansion of the blastocoele cavity (B1–B6), inner cell mass (type A/B/C), and trophectodermal cells (type A/B/C) [1]. Blastocysts filled B3–B6, inner cell mass, and trophectoderm layer scored A or B were transferred or cryopreserved on day 5 or day 6. Usually, the two best-quality embryos were transferred on day 3 or day 5. Additional viable embryos were cryopreserved for the subsequent FET cycles. High progesterone concentration (> 2.0 ng/ml on the day of HCG administration or other factors that were not conducive to embryo implantation) of the fresh embryo transfer was cancelled to prevent the occurrence of severe ovarian hyperstimulation syndrome.

Embryo vitrification and warming

The methods for embryo vitrification and warming were previously described [20,21], and the vitrification procedures were performed at room temperature. Embryos were first equilibrated in 1 ml of equilibration solution for 5 min, transferred to 1 ml of vitrification solution, and loaded onto the Cryotop within 1 min. Embryos were then immediately loaded onto the surface of the Cryotop with a minimum volume. Typically, two embryos were loaded onto one Cryotop, and different color Cryotops were used to mark the embryo quality.

Embryos were warmed on the day of embryo transfer, and the plastic cover was removed in the liquid nitrogen. The end of the Cryotop was directly immersed into 1 ml of 37 °C TS (1.0 mol/L sucrose solution) for 1 min, transferred into 1 ml of DS (0.5 mol/L sucrose solution) for 3 min at room temperature, and then washed twice in base medium for 5 min at room temperature. Embryos were observed 2 h after warming by using an inverted microscopy. The cleavage-stage embryos with more than half of the blastomeres surviving the re-expansion were evaluated for survival.

Assessment of pregnancy

Serum HCG was measured 2 weeks later. A clinical pregnancy was confirmed when a fetal heartbeat was identified via ultrasound 4 weeks after the transfer. Live birth was confirmed by hospital records or telephone interviews of the couples. Patients failing to achieve

clinical pregnancy after fresh embryo transfer went through frozen and thaw cycles until all vitrified embryos were transferred or a live birth was achieved. Cumulative clinical pregnancy was defined as clinical pregnancy achieved from a retrieved oocyte cycle. The primary outcome measure was the cumulative clinical pregnancy rate (CPR) and cumulative live birth rate (CBR).

Statistical analysis

Data were analyzed using SPSS version 22.0. All tests were two-tailed, and statistical significance was defined as a P-value < 0.05. Basal characteristics and ovarian stimulation outcomes were compared between the day 2/3 and day 5/6 groups by using chi-square or Fisher's exact tests for categorical variables and student t-tests for continuous variables.

This work is a retrospective study. The patients were not randomly allocated, with the potential confounding and selection biases influencing the outcomes. Thus, we used propensity score matching to identify day 2/3 group women who were most identical to the day 5/6 group women. Propensity scores were assigned to the basal characteristics and ovarian stimulation variables that potentially affect the outcomes. We used a logistic regression model to estimate the propensity scores using the following characteristics as covariates in the model: female age, basal FSH, peak E2 value, P value on hCG day, number of oocytes retrieved, number of mature oocytes, number of fertilized oocytes, and number of cleavage embryos. A one-to-one match between the day 5/6 group and day 2/3 group was subsequently obtained using nearest neighbor matching, with a caliper width of 0.01 and without replacement.

Meta-analysis

A systematic meta-analysis was conducted to further analyze the effects of day 5/6 transfer on the CPR of patients. A comprehensive review of the literature was conducted using the search terms "day 2 or day two," "day 3 or day three," "cleavage*," "day 5 or day five," "day 6 or day six," "blastocyst*," "embryo* transfer*," and "pregnancy" in PubMed and Embase (the last search was performed on April 15, 2016). Only the studies that compared the cumulative outcomes (fresh and frozen transfers) of cleavage embryo transfer and blastocyst embryo transfer in IVF cycles were included. Searches were restricted to articles published in English. The articles were excluded if no extractable data were present. Such studies will only be briefly described in this paper. The meta-analysis was performed using the RevMan 5.3 software.

Results

A total of 1051 infertile patients were included in this study. The day 2/3 and 5/6 groups consisted of 834 and 217 patients, respectively. After propensity score matching was performed, the day 2/3 group consisted of 217 patients who matched the patients in the day 5/6 group. The baseline characteristics, ovarian stimulation outcomes, and clinical outcomes before and after matching were evaluated. The study design is shown in Fig. 1.

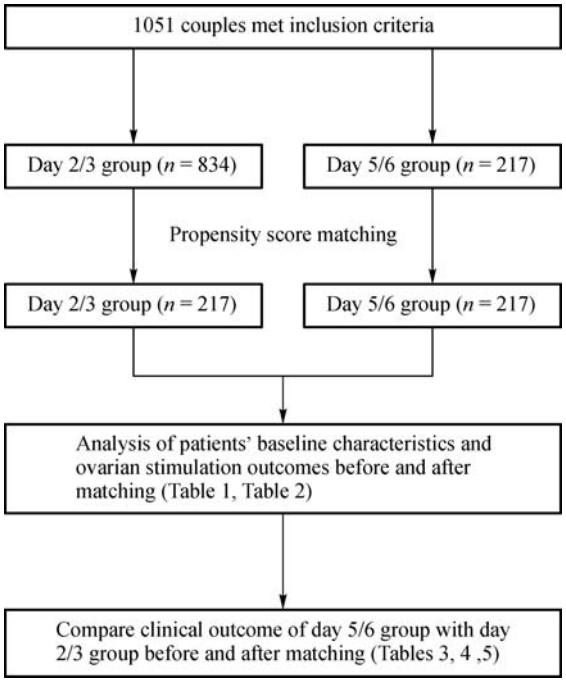

Fig. 1 Study design.

Patient characteristics and ovarian stimulation outcomes

Before matching, the patients in the day 2/3 group were older (31.75 ± 4.65 years vs. 30.86 ± 4.60 years, P = 0.012) and had higher basal FSH (7.24 ± 2.09 vs. 6.67 ± 1.73, $P < 0.001$). The baseline patient characteristics before and after the propensity score matching are summarized in Table 1. After matching, no significant difference was observed between the day 2/3 and 5/6 groups as shown in Table 1.

The ovarian stimulation outcomes are summarized in Table 2. The day 2/3 group patients showed a lower peak E2 value (4056 ± 2509 vs. 4709 ± 2320, P = 0.001), fewer retrieved oocytes (13.10 ± 5.23 vs. 15.87 ± 5.25, $P < 0.001$), fewer mature oocytes (11.15 ± 3.35 vs. 13.04 ± 4.15, $P < 0.001$), fewer 2PN (7.66 ± 4.82 vs. 9.42 ± 5.62, $P < 0.001$), and fewer cleavage stage embryos (4.75 ± 3.82 vs. 5.87 ± 3.91, $P < 0.001$) than

Table 1 Baseline characteristics of patients before and after the propensity score matching

Characteristics	Day 5/6 group	Before matching		After matching	
		Day 2/3 group	*P* value	Day 2/3 group	*P* value
No. of patients	217	834		217	
No. of cycles (mean±SD)	1.28±0.652	1.26±0.542	0.570	1.29±0.566	0.814
Female age (year, mean±SD)	30.86±4.60	31.75±4.65	0.012	30.19±4.15	0.113
Basal FSH (mIU/ml, mean±SD)	6.67±1.73	7.24±2.09	<0.001	6.58±1.47	0.562
Duration of infertility (year, mean±SD)	4.7±3.2	5.0±3.9	0.27	4.9±3.9	0.48
Primary infertility (%)	51.5	51.4	0.35	51.6	0.923
Diagnosis					
Tubal factor (%)	40.0	43.9	0.95	44.0	0.437
Anovulation (%)	5.5	5.8	0.89	6.0	0.84
Male factor (%)	20.0	14.7	0.068	15.0	0.20
Idiopathic (%)	6.4	4.5	0.25	5.0	0.53
Mix (%)	28.2	31.0	0.42	30	0.67

Day 2/3 group, underwent cleavage-stage embryo transfer on day 2/3; day 5/6 group, underwent blastocyst stage embryo transfer on day5/6; No., number.

Table 2 Ovarian stimulation outcomes of patients before and after the propensity score matching

Characteristics	Day 5/6 group	Before matching		After matching	
		Day 2/3 group	*P* value	Day 2/3 group	*P* value
E2 value on hCG day	4709±2320	4056±2509	0.001	4548±2302	0.469
P value on hCG day	1.20±0.47	1.14±0.64	0.207	1.16±0.52	0.496
No. of oocytes retrieved	15.87±5.25	13.10±5.23	<0.001	14.93±5.12	0.062
No. of mature oocytes	13.04±4.15	11.15±3.35	<0.001	12.85±3.22	0.594
No. of 2PN	9.42±5.62	7.66±4.82	<0.001	8.62±4.41	0.100
No. of embryos	5.87±3.91	4.75±3.82	<0.001	5.32±3.71	0.076
No. of vitrified embryos	2.73±2.41	2.27±2.19	0.011	2.78±2.21	0.803

Values are mean±SD. Day 2/3 group, underwent cleavage-stage embryo transfer on day 2/3; day 5/6 group, underwent blastocyst stage embryo transfer on day 5/6; No., number.

the day 5/6 patients. The blastocyst formation rate was 63.0%, and the viable blastocyst formation rate was 48.5%. After the propensity score matching, the ovarian stimulation outcomes of the day 2/3 group were similar to those of the day 5/6 group.

Clinical outcomes of patients in the day 2/3 group and day 5/6 group

We compared the clinical outcomes of patients who received day 2/3 embryo transfer versus patients who underwent day 5/6 blastocyst transfer. Table 3 shows that the day 5/6 group in fresh cycles had higher clinical pregnancy rate (50% vs. 46.42%, $P = 0.557$; 50% vs. 47.26%, $P = 0.698$), live birth rate (46.05% vs. 39.61%, $P = 0.284$; 46.05% vs. 40.41%, $P = 0.420$) and lower abortion rate (7.89% vs. 12.78%, $P = 0.394$; 7.89% vs. 13.04%, $P = 0.424$) than the 2/3 group before and after matching, but these differences were not significant.

The frozen cycle clinical outcome is summarized in Table 4. The embryo survival rate in freeze–thaw cycles was similar between the two groups. However, the day 5/6 group showed significantly higher pregnancy rate (53.80% vs. 35.67%, $P < 0.001$; 53.80% vs. 42.50%, $P < 0.001$) and live birth rate (46.78% vs. 25.15%, $P < 0.001$; 46.78% vs. 30.63%, $P < 0.001$) than the day 2/3 group before and after the matching of the frozen cycles.

As shown in Table 5, the day 5/6 group had a higher CPR (57.14% vs. 53.46%, OR 1.16, 95% CI 0.79–1.70) and CBR (53.00% vs. 49.77%, OR 1.14, 95% CI 0.78–1.66) than the day 2/3 group, although this difference was not statistically significant. Moreover, both the mean cycles per live birth (1.11 ± 0.32 vs. 1.50 ± 0.65, $P < 0.001$) and mean days per live birth (336.33 ± 60.07 vs. 373.67 ± 129.96, $P = 0.006$) in the day 5/6 group were significantly lower than those in the day 2/3 group.

Meta-analysis

A systematic meta-analysis was conducted to further investigate the effects of day 5/6 on the CPR of patients. After screening the titles and abstracts, 52 full manuscripts were retrieved for detailed evaluation. Eight articles met our criteria for meta-analysis (including the present study). The selection flow chart is shown in Supplementary Fig. S1. Our meta-analysis showed no significant difference in

Table 3 Clinical outcome of patients in fresh cycle before and after the propensity score matching

Characteristics	Day 5/6 group	Before matching		After matching	
		Day 2/3 group	*P* value	Day 2/3 group	*P* value
No. of patients	76	573		146	
No. of embryos transferred (mean±SD)	1.89±0.5	1.99±0.38	0.301	2.00±0.4	0.081
PR per transfer cycle, *n* (%)	38 (50.00)	266 (46.42)	0.557	69 (47.26)	0.698
BR per transfer cycle, *n* (%)	35 (46.05)	227 (39.61)	0.284	59 (40.41)	0.420
Abortion rate, *n* (%)	3 (7.89)	34 (12.78)	0.394	9 (13.04)	0.424
Ectopic pregnancy rate, *n* (%)	0 (0)	5 (1.88)	0.746	1 (1.45)	0.751

Day 2/3 group, underwent cleavage-stage embryo transfer on day 2/3; day 5/6 group, underwent blastocyst stage embryo transfer on day5/6; No., number; PR, pregnancy rate; BR, live birth rate.

Table 4 Clinical outcome of patients in frozen cycles before and after the propensity score matching

Characteristics	Day 5/6 group	Before matching		After matching	
		Day 2/3 group	*P* value	Day 2/3 group	*P* value
No. of patients	148	508		112	
No. of transfer cycles	171	684		160	
Embryo survival rate, *n*/*n* (%)	332/342 (97.08)	1340/1380 (97.10)	0.980	312/321 (97.20)	0.926
PR per transfer cycle, *n* (%)	92 (53.80)	244 (35.67)	<0.001	68 (42.50)	<0.001
BR per transfer cycle, *n* (%)	80 (46.78)	172 (25.15)	<0.001	49 (30.63)	<0.001

Day 2/3 group, underwent cleavage-stage embryo transfer on day 2/3; day 5/6 group, underwent blastocyst stage embryo transfer on day5/6; No., number; PR, pregnancy rate; BR, live birth rate.

Table 5 Cumulative clinical outcomes of patients before and after the propensity score matching

Characteristics	Day 5/6 group	Before matching		After matching	
		Day 2/3 group	*P* value	Day 2/3 group	*P* value
No. of patients	217	834		217	
CPR, *n* (%)	124 (57.14)	434 (52.04)	0.180	116 (53.46)	0.440
CBR, *n* (%)	115 (53.00)	399 (47.84)	0.177	108 (49.77)	0.502
Mean cycles per live birth (mean±SD)	1.11±0.32	1.32±0.55	<0.001	1.50±0.65	<0.001
Mean days per live birth (mean±SD)	336.33±60.07	358.13±105.66	0.035	373.67±129.96	0.006

Day 2/3 group, underwent cleavage-stage embryo transfer on day 2/3; day 5/6 group, underwent blastocyst stage embryo transfer on day5/6; No., number; CPR, cumulative pregnancy rate; CBR, cumulative live birth rate.

the cumulative live birth rate between the two groups (OR 0.89, 95% CI 0.58–1.36). High heterogeneity was detected, and the I^2 was 69% (Supplementary Fig.S2). The shapes of the funnel plot of the cumulative live birth rate between the two groups did not reveal any evidence of asymmetry (Supplemental Fig.S3). The meta-analysis also showed no significant difference in the cumulative pregnancy rate between the day 5/6 and day 2/3 groups (OR 1.03, 95% CI 0.69–1.53). Higher heterogeneity was detected, and the I^2 was 67% (Supplementary Fig.S4). The shapes of the funnel plot showed evident asymmetry (Supplementary Fig.S5).

Discussion

In this study, we compared the cumulative outcomes of day 5/6 embryo transfer and day 2/3 embryo transfer on couples treated for infertility. Through a propensity score-matched study and meta-analysis, we found that the day 5/6 group had a higher cumulative live birth rate and cumulative pregnancy rate than the day 2/3 group, although the difference was not statistically significant. Moreover, we found that the day 5/6 group had a lower mean cycle per live birth and mean days per live birth according to the retrospective study. Therefore, day 5/6 embryo transfer is a more cost-effective, time efficient policy for selecting viable embryos to obtain a live baby.

Previous studies compared the fresh cycle outcomes between the day 5/6 and day 2/3 groups. Several studies reported a higher clinical pregnancy rate and live birth rate with day 5/6 embryos transfer in different groups of couples [19,22]; however, some studies did not confirm these results [23–25]. In our study, couples in the day 5/6 group had higher clinical pregnancy rate and live birth rate in fresh cycles with an equal number of embryo transfer; however, the differences did not reach statistical significance. Increasing the number of patients in the day 5/6 group might allow us to observe a significant difference.

The data from this study showed higher PR and BR per frozen transfer cycle after day 5/6 embryo transfer. The results are contradictory to those of previous studies that reported a similar PR or BR in the frozen cycles between the two treatment groups. This discrepancy might be due to the freeze–thaw size. The sample sizes of the day 5/6 group in the studies of Rienzi *et al*. [24] and Fernández-Shaw *et al*. [25] were 8 and 24, respectively; however, our study had a larger sample size (171 in day 5/6 group and 160 in day 2/3 group). The verification protocol with a higher embryo survival rate in our study might also contribute to these results.

In our study, day 5/6 embryo transfer led to a higher cumulative pregnancy rate and cumulative live birth rate than day 2/3 embryo transfer, although the difference was not statistically significant. These results were unchanged after conducting the meta-analysis. The results are not in consensus with a systematic review [8] that favored day 2/3 embryo transfer regarding the cumulative pregnancy rate. Considering the similar blastocyst formation rate (from 44.7% to 48.3% in reviewed articles vs. 48.5% in our study), the difference might result from the frozen and thaw protocols. Slow freezing was used in the reviewed articles, and the blastocyst survival rate ranged from 5.6% to 53.2% [23–25]. All of the cycles in our study used a verification protocol with a higher embryo survival rate (97.08% in day 5/6 group and 97.20% in day 2/3 group). Racial differences might also contribute to the discrepancy in the cumulative outcomes between the previous studies and our current study.

Despite the cumulative outcomes, the mean cycles per live birth and mean days per live birth in the day 5/6 group were lower than those in the day 2/3 group. Therefore, day 5/6 transfer costs less time and expense to produce a live baby. One possible reason is that the blastocyst culture is a more effective method in selecting viable embryos.

This study has several limitations. First, the retrospective nature of this study led to potential selection bias in our population. However, this bias was limited by the similar baseline characteristics between the two groups after the propensity score matching analysis. Second, our study excluded couples who had less than 3 or greater than 20 cleavage stage embryos due to our center situation. However, individuals who had less than 3 cleavage stage embryos would not receive blastocyst culture and had no significant differences regarding the cumulative outcomes. Individuals who had more than 20 embryos cannot receive day 2/3 embryo transfer and had no comparable match between the two groups. Third, most patients in day 5/6 group had frozen cycles with higher PR and BR than those in day 2/3 group, whereas most patients in day 2/3 group had fresh cycles with a similar PR and BR in fresh cycles. Thus, CPR and CBR might be affected by the difference in fresh and frozen embryo transplantation.

Our propensity score-matched study and meta-analysis revealed that the cumulative live birth rate and cumulative pregnancy rate of the day 5/6 group were higher than those of the day 2/3 group, although this difference was not significant. Day 5/6 embryo transfer is a more cost- and time-efficient policy than day 2/3 embryo transfer in terms of the production of a live baby.

Acknowledgements

This work was supported by the Ministry of Health Public Foundation of China (No.201402004), National Basic Research Program of China (973 Program, No. 2007CB948104), Huazhong University of Science and Technology Independent Innovation Research Fund (No. 2013ZHYX010), Specialized Research Fund for the Commission of Hubei Province (No. JS-2011002), and National Natural Science Foundation of China (No. 81601348).

Compliance with ethics guidelines

Ye Yin, Ge Chen, Kezhen Li, Qiuyue Liao, Sijia Zhang, Nieying Ma, Jing Chen, Yan Zhang, and Jihui Ai declare no conflict of interest with regard to this work. All included procedures were conducted in accordance with the ethical standards of the Responsible Committee on Human Experimentation (institutional and national) and with the *Helsinki Declaration* of 1975, as revised in 2000 (5). Informed consent was obtained from all patients upon enrollment to the study.

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s11684-017-0535-6 and is accessible to authorized users.

References

1. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in *in-vitro* fertilization. Hum Reprod 1998; 13(12): 3434–3440
2. Hsieh YY, Tsai HD, Chang FC. Routine blastocyst culture and transfer: 201 patients' experience. J Assist Reprod Genet 2000; 17 (8): 405–408
3. Van der Auwera I, Debrock S, Spiessens C, Afschrift H, Bakelants E, Meuleman C, Meeuwis L, D'Hooghe TM. A prospective randomized study: day 2 versus day 5 embryo transfer. Hum Reprod 2002; 17(6): 1507–1512
4. Smith LP, Oskowitz SP, Dodge LE, Hacker MR. Risk of ectopic pregnancy following day-5 embryo transfer compared with day-3 transfer. Reprod Biomed Online 2013; 27(4): 407–413
5. Huang B, Hu D, Qian K, Ai J, Li Y, Jin L, Zhu G, Zhang H. Is frozen embryo transfer cycle associated with a significantly lower incidence of ectopic pregnancy? An analysis of more than 30,000 cycles. Fertil Steril 2014; 102(5): 1345–1349
6. Alper MM, Brinsden P, Fischer R, Wikland M. To blastocyst or not to blastocyst? That is the question. Hum Reprod 2001; 16(4): 617–619

7. Tsirigotis M. Blastocyst stage transfer: pitfalls and benefits. Too soon to abandon current practice? Hum Reprod 1998; 13(12): 3285–3289
8. Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev 2012; 7(7): CD002118
9. Barnhart KT. Introduction: are we ready to eliminate the transfer of fresh embryos in *in vitro* fertilization? Fertil Steril 2014; 102(1): 1–2
10. Lintsen AME, Braat DDM, Habbema JDF, Kremer JAM, Eijkemans MJC. Can differences in IVF success rates between centres be explained by patient characteristics and sample size? Hum Reprod 2010; 25(1): 110–117
11. Bodri D, Kawachiya S, De Brucker M, Tournaye H, Kondo M, Kato R, Matsumoto T. Cumulative success rates following mild IVF in unselected infertile patients: a 3-year, single-centre cohort study. Reprod Biomed Online 2014; 28(5): 572–581
12. Trokoudes KM, Pavlides C, Zhang X. Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program. Fertil Steril 2011; 95(6): 1996–2000
13. Cobo A, de los Santos MJ, Castellò D, Gámiz P, Campos P, Remohí J. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil Steril 2012; 98(5): 1138–1146.e1
14. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, Tarlatzis BC. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil Steril 2008; 90(1): 186–193
15. AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. Reprod Biomed Online 2010; 20(2): 209–222
16. Gleicher N, Kushnir VA, Barad DH. Is it time for a paradigm shift in understanding embryo selection? Reprod Biol Endocrinol 2015; 13 (1): 3
17. Xi QS, Zhu LX, Hu J, Wu L, Zhang HW. Should few retrieved oocytes be as an indication for intracytoplasmic sperm injection? J Zhejiang Univ Sci B 2012; 13(9): 717–722
18. Yu Z, Dong X, Rui W, Wei Y, Zhang H, Zhu G, Ai J. The criteria for optimal down-regulation with gonadotropin-releasing hormone-agonist: a retrospective cohort study. Gynecol Endocrinol 2015; 31 (12): 959–965
19. Papanikolaou EGD, D'haeseleer E, Verheyen G, Van de Velde H, Camus M, Van Steirteghem A, Devroey P, Tournaye H. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study. Hum Reprod 2005; 20(11): 3198–3203
20. Yu Z, Dong X, Wei Y, Zhang H, Ai J. The artificial cycle method improves the pregnancy outcome in freeze–thawed embryo transfer: a retrospective cohort study. Gynecol Endocrinol 2015; 31(1): 70–74
21. Huang B, Ren X, Wu L, Zhu L, Xu B, Li Y, Ai J, Jin L. Elevated progesterone levels on the day of oocyte maturation may affect top quality embryo IVF cycles. PLoS One 2016; 11(1): e0145895
22. Elgindy EA, Abou-Setta AM, Mostafa MI. Blastocyst-stage versus cleavage-stage embryo transfer in women with high oestradiol concentrations: randomized controlled trial. Reprod Biomed Online 2011; 23(6): 789–798
23. Emiliani S, Delbaere A, Vannin AS, Biramane J, Verdoodt M, Englert Y, Devreker F. Similar delivery rates in a blastocyst cryopreservation to optimize outcomes of warming cycles 159 selected group of patients, for day 2 and day 5 embryos both cultured in sequential medium: a randomized study. Hum Reprod 2003; 18(10): 2145–2150
24. Rienzi L, Ubaldi F, Iacobelli M, Ferrero S, Minasi MG, Martinez F, Tesarik J, Greco E. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. Hum Reprod 2002; 17(7): 1852–1855
25. Fernández-Shaw S, Cercas R, Braña 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(2): 177–184