Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan

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Objective: To evaluate the relationship between frozen-thawed single blastocyst transfer (BT) and maternal and neonatal outcomes of pregnancy.
Design: Retrospective analysis.
Setting: Japanese nationwide registry of assisted reproductive technology (ART) with mandatory reporting for all ART clinics in Japan.
Patient(s): Registered from 2008 through 2010 undergoing single embryo transfer cycles (n = 277,042).
Intervention(s): None.
Main Outcome Measure(s): Rates of preterm birth (PTB; <37 weeks' gestation), low birth weight (LBW; <2,500 g), small for gestational age (SGA), large for gestational age (LGA), placenta previa, placenta abruption, placenta accreta, and pregnancy-induced hypertension (PIH) after fresh/frozen-thawed and cleaved-embryo/blastocyst transfers were performed.
Result(s): Frozen-thawed embryo transfer (FET) was associated with a significantly reduced occurrence of PTB, LBW, and SGA but increased rate of LGA. FET was also associated with a higher incidence of placenta accreta (odds ratio 3.16) and PIH (odds ratio 1.58). BT was associated with a significantly decreased rate of SGA and increased rate of LGA. There was no significant association between BT and maternal complications.
Conclusion(s): Frozen-thawed BT is associated with improved general perinatal outcomes of pregnancy but significantly increased maternal risks of placenta accreta and PIH. This finding requires further investigation to assure maternal safety of patients undergoing ART treatment. (Fertil Steril® 2014;101:128–33. ©2014 by American Society for Reproductive Medicine.)
Key Words: Frozen-thawed blastocyst transfer, placenta accreta, pregnancy-induced hypertension

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The transition to single-embryo transfer (SET) has become the central strategy to realize high-quality safe assisted reproductive technology (ART) practice because SET is able to minimize the occurrence of multiple pregnancies (1, 2). At the same time, newly introduced technologies,

e.g., frozen-thawed embryo transfer (FET) and blastocyst transfer (BT), are practiced with the intention to improve cumulative pregnancy rates for each patient without extra multiples. It is a necessary consequence to assess the safety of these new technologies regarding maternal and neonatal outcomes. A number of valuable original papers (3–6), reviews, and meta-analyses (7, 8) have been published. However, it is very difficult to compare various outcomes after ART with spontaneously conceived patients owing to the difference of populations and various confounding variables. Moreover, it would require a large number of well controlled studies to describe safety issues, especially given the relative infrequency of adverse events during the perinatal period.

We previously reported, with the use of data from 20,886 clinical pregnancies after SET in the 2008 Japanese registry database, that frozen-thawed BT is associated with a reduced ectopic pregnancy risk compared with fresh cleaved embryo transfer (9). Because endometrial receptivity and placentation are possibly key factors in the prevalence of ectopic pregnancy, it seems to be appropriate to investigate other placenta-associated complications as potentially relevant factors during pregnancy. However, there were not a sufficient number of cycles in the registry to study the possible impact of frozen-thawed BT on relatively infrequent events and complications during pregnancy. In the present paper, we combined three consecutive years of the Japanese ART registry database from 2008 through 2010 to assess the potential association of selected perinatal and maternal outcomes regarding the comparison of frozen-thawed transfer cycle pregnancies with fresh transfer pregnancies and the comparison of single-blastocyst transfer cycle pregnancies with cleaved embryo transfer pregnancies.

MATERIALS AND METHODS

The data analyzed in this study are part of the Japanese ART registry database collected by the Japan Society of Obstetrics and Gynecology (JSOG) on a mandatory basis from all ART clinics through secure internet access in 2008 (n = 609), 2009 (n = 625), and 2010 (n = 591), as previously described in detail (9, 10). The dataset consists of cycle-specific data and certain outcomes of treatment as well as the fate of pregnancy and obstetrical outcome data. The population in this study includes fresh ET cases of IVF and intracytoplasmic sperm injection (ICSI) as well as FET from 2008 through 2010. All of the embryos transferred were autologous for couples, because donor gametes or embryos are not allowed to be used in ART in Japan. In addition, only the data of SET cycles were provided for the study, because the number of transferred embryos may affect the outcome of pregnancy and complications even if singleton pregnancy is selected for the final analysis. SET cycles constituted >70% of the total ET cycles during the period because since 2008 JSOG guidelines have made it mandatory for patients <35 years of age to undergo SET for the first and second ETs to reduce the multiple pregnancy rate.

Small for gestational age (SGA) and large for gestational age (LGA) were defined as >2 standard deviations below or above the Japanese gestational age- and gender-specific growth standard published by the Japan Pediatric Society (11). Placenta previa, placenta abruption, placenta accreta, and pregnancy-induced hypertension (PIH) were diagnosed at each clinic and described in a webpage section. These descriptive data were electronically identified and presented as numbers and percentages. Risk factor analysis for preterm birth (PTB; <37 weeks' gestation), low birth weight (LBW; <2,500 g), SGA, LGA, placenta previa, placenta abruption, placenta accreta, and PIH was performed by multivariable logistic regression analysis. Variables extracted for this study included maternal age at the time of ART treatment, whether a frozen-thawed or a fresh embryo was transferred, whether a blastocyst or a cleaved embryo was transferred, and the sex of the infant. These independent variables were selected by means of backward elimination in each analysis. Crude odds ratio (COR) and adjusted odds ratio (AOR) with 95% confidence intervals (CIs) and *P* values of likelihood ratio test were calculated with the use of SAS software JMP version 10.0.2 and SAS version 9.1.3 SP4 (SAS Institute).

This study was planned by the registration and research subcommittee of the JSOG Ethics Committee. The dataset was provided as an Excel file for the research proposal after review and approval by the JSOG Board of Ethics, which is an independent reviewing body for research and investigational proposals. Institutional Review Board approval was not obtained, because the dataset was collected by JSOG from clinics and the data do not include any personally identifiable parameters.

RESULTS

For the period from 2008 through 2010, 277,042 SETs were registered in the JSOG database (Table 1). Even though the average maternal age was 36.3 ± 4.5 years and one-fourth of the cycles were for patients aged >40 years, 79,626 cycles (28.7%) resulted in clinical pregnancy, as defined by the International Committee Monitoring Assisted Reproductive Technologies (ICMART) glossary, following SET (12). Because some cases were lost to follow-up during the early stage of pregnancy, we knew pregnancy outcome in 93.6% of the cycles (74,539/79,626). The follow-up rates were similar for all subgroups: fresh cleaved 94.3%, fresh blastocyst 92.4%, thawed cleaved 92.1%, and thawed blastocyst 93.8%. We extracted the 53,023 singleton ongoing pregnancies (>21 weeks of gestation) for further analysis. Detailed obstetrical and neonatal information were available for 90.8% of the extracted cycles (48,158/53,023) and are described as numbers and percentages in Table 2. We used 48,158 cycles for the analysis of different variables.

Risk for Preterm Birth and Birth Weight of Infant

In Table 3 advanced maternal age is associated with both PTB before 37 weeks (AOR 1.02 [95% CI 1.01–1.03]) and LBW <2,500 g (AOR 1.01 [95% CI 1.00–1.02]). The AOR for PTB before 37 weeks was lower in FET (AOR 0.90 [95% CI 0.82–0.98]) as were the odds of LBW <2,500 g was lower (AOR 0.71 [95% CI 0.66–0.77]). BT did not have significant effects on these two parameters. Advanced maternal age

TABLE 1

Maternal age and the outcome of pregnancies after single embryo transfers (SETs) in Japan 2008–2010.

	Fresh cleaved	Fresh blastocyst	Thawed cleaved	Thawed blastocyst	Total
No. of SET cycles	91,485	33,559	33,132	118,866	277,042
Maternal age (y), n (%)					
Mean (SD)	37.0 (4.6)	35.8 (4.3)	36.3 (4.7)	36.0 (4.2)	36.3 (4.5)
<30	5,093 (5.6)	2,602 (7.8)	2,338 (7.1)	8,196 (6.9)	18,229 (6.6)
30–34	22,928 (28.3)	10,415 (25.1)	9,782 (31.0)	35,270 (29.5)	78,395 (28.3)
35–39	35,149 (40.2)	13,741 (38.4)	12,531 (40.9)	49,934 (37.8)	111,355 (40.2)
≥40	28,315 (24.9)	6,801 (31.0)	8,481 (20.3)	25,466 (25.6)	69,093 (24.9)
No. of pregnancies (% of pregnancy/ET)	17,354 (19.0)	10,063 (30.0)	6,441 (19.4)	45,767 (38.5)	79,625 (28.7)
Reported outcome of the pregnancy excluding unknown cycles, n (%)					
Total	16,365	9,302	5,930	42,942	74,539
Live birth	11,802 (72.1)	6,883 (74.0)	4,232 (71.4)	30,952 (72.1)	53,869 (72.3)
Ectopic	291 (1.8)	137 (1.5)	79 (1.3)	268 (0.6)	775 (1.0)
Miscarriage	4,155 (25.4)	2,208 (23.7)	1,580 (26.6)	11,405 (26.6)	19,348 (26.0)
Abortion	37 (0.2)	23 (0.2)	21 (0.4)	147 (0.3)	228 (0.3)
Stillbirth	80 (0.5)	51 (0.5)	18 (0.4)	170 (0.3)	319 (0.3)
Reported no. (%) of neonates excluding unknown cycles					
Singleton	11,735 (67.6)	6,743 (67.0)	4,170 (64.7)	30,375 (66.4)	53,023 (66.6)
Twin	90 (0.5)	136 (1.4)	49 (0.8)	450 (1.0)	725 (0.9)
Triplet or more	2 (0.01)	2 (0.02)	0 (0)	5 (0.01)	9 (0.01)

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was not associated with the odds of SGA, but it was associated with higher odds of LGA births. FET was associated with significantly lower odds of SGA (AOR 0.67 [95% CI 0.60–0.75]), as was BT (AOR 0.83 [95% CI 0.74–0.92]). In addition, the odds of LGA were significantly higher with FET (AOR 1.48 [95% CI 1.38–1.58]) and BT (AOR 1.14 [95% CI 1.07–1.23]).

Risk for Maternal Perinatal Complications

In Table 4 advanced maternal age was associated with significantly increased odds of both placenta previa (AOR 1.05 [95% CI 1.02–1.08]) and PIH (AOR 1.07 [95% CI 1.06–1.09]). FET was associated with significantly higher odds of both placenta accreta (AOR 3.16 [95% CI 1.71–6.23]) and PIH

TABLE 2

Obstetrical and neonatal outcomes and events in singleton live birth pregnancies after single embryo transfers in Japan during 2008–2010.

	Fresh cleaved	Fresh blastocyst	Thawed cleaved	Thawed blastocyst	Total
Information available (%)	10,928 (22.7)	5,981 (12.4)	3,841 (8.0)	27,408 (56.9)	48,158 (100)
Gestational age (wk), n (%)					
Mean (SD)	38.6 (2.0)	38.5 (2.0)	38.8 (1.9)	38.7 (2.0)	38.7 (2.0)
37–41	10,128 (92.7)	5,496 (91.9)	3,586 (93.4)	25,403 (92.7)	44,613 (92.6)
<37	661 (6.1)	403 (6.7)	210 (5.5)	1,656 (6.0)	2,930 (6.1)
<32	78 (0.71)	42 (0.70)	32 (0.83)	196 (0.72)	348 (0.72)
<28	61 (0.56)	40 (0.67)	13 (0.34)	153 (0.56)	267 (0.55)
Neonatal weight (g), n (%)					
Mean (SD)	2,940 (470)	2,947 (480)	3,020 (456)	3,047 (480)	3,008 (478)
<1,500	152 (1.4)	85 (1.4)	38 (1.0)	338 (1.2)	613 (1.3)
<2,500	1,329 (12.2)	758 (12.7)	369 (9.6)	2,468 (9.0)	4,924 (10.2)
>4,000	66 (0.60)	44 (0.74)	53 (1.38)	408 (1.49)	571 (1.19)
>4,500	7 (0.06)	4 (0.07)	3 (0.08)	25 (0.09)	39 (0.08)
SGA (%)	702 (6.4)	357 (6.0)	194 (5.1)	1,041 (3.8)	2,294 (4.8)
LGA (%)	1,263 (11.6)	741 (12.4)	587 (15.3)	4,989 (18.2)	7,580 (15.7)
Gender of neonate					
Male	5,438	3,150	1,924	14,710	25,222
Female	5,433	2,798	1,900	12,547	22,673
Unknown	57	38	17	151	263
Sex ratio (male/female)	1.00	1.13	1.01	1.17	1.11
Obstetrical events					
Cesarean section (%)	3,026 (27.7)	1,682 (28.1)	1,322 (34.4)	9,941 (36.3)	15,971 (33.2)
Placenta previa (%)	96 (0.88)	48 (0.80)	35 (0.91)	203 (0.74)	382 (0.79)
Placenta abruption (%)	36 (0.33)	16 (0.27)	11 (0.29)	46 (0.17)	109 (0.23)
Placenta accreta (%)	10 (0.09)	5 (0.08)	10 (0.26)	73 (0.27)	98 (0.20)
PIH (%)	229 (2.1)	87 (1.5)	102 (2.7)	794 (2.9)	1,212 (2.5)

Note: LGA = large for gestational age; PIH = pregnancy-induced hypertension; SGA = small for gestational age.

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TABLE 3

Odds ratios of preterm birth (< 37 weeks' gestation), low birth weight (< 2,500 g), SGA, and LGA according to frozen-thawed compared with fresh and blastocyst compared with cleaved embryo among singleton pregnancies after single-embryo transfer.

	COR	AOR	95% CI	*P* value	COR	AOR	95% CI	*P* value
Outcome			**< 37 wk**				**< 2,500 g**	
Maternal age (per/y)	1.02	1.02	1.01–1.03	< .001	1.01	1.01	1.00–1.02	< .001
Frozen-thawed/fresh	0.95	0.90	0.82–0.98	.02	0.71	0.71	0.66–0.77	< .001
Blastocyst/cleaved embryo	1.04	1.09	0.99–1.20	.07	0.80	0.97	0.90–1.05	.51
Male/female	1.14	1.14	1.06–1.23	< .001	0.75	0.76	0.71–0.81	< .001
Outcome			**SGA**				**LGA**	
Maternal age (per/y)	0.99	0.99	0.98–1.01	.26	1.02	1.03	1.02–1.04	< .001
Frozen-thawed/fresh	0.61	0.67	0.60–0.75	< .001	1.68	1.48	1.38–1.58	< .001
Blastocyst/cleaved embryo	0.67	0.83	0.74–0.92	< .001	1.55	1.14	1.07–1.23	< .001
Male/female	0.76	0.77	0.71–0.85	< .001	1.09	1.08	1.03–1.14	.003

Note: Odds ratios obtained by multiple logistic regression analysis including all variables listed in the column. CI = confidence interval; COR = crude odds ratio; AOR = adjusted odds ratio; other abbreviations as in Table 2.

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(AOR 1.58 [95% CI 1.35–1.86]). BT was not associated with the odds of the four selected placenta-associated complications during pregnancy: placenta previa, placenta abruption, placenta accreta, and PIH.

DISCUSSION

The ICMART world report for 2004, the most recent and the most comprehensive ART worldwide data, showed that multiple birth rates due to ART had considerably decreased in response to a reduced number of embryos transferred (13). In response to this change of clinical practice that leads to a greater number of embryos being frozen, the proportion of FET that was conducted relative to the total initiated cycles in the same year was 31%. It has also been reported that the cumulative live birth rate of single fresh ET followed by single FET resulted in a not statistically different effectiveness compared with fresh double-embryo transfer (1). In addition, there have been several recent studies that suggested better outcome and safety of FET (6–8), although other papers have suggested possible adverse effects of FET on obstetrical outcome, including a higher rate of preeclampsia (14).

On the other hand, BT has become increasingly used to achieve higher implantation rates since improved laboratory standards and refined culture media have made universal BT feasible. However, the suggested resulting perinatal outcome after BT is still conflicting, especially for a possible increase of PTB (4, 5, 15). In addition, there have been several reports suggesting altered sex ratio toward more males by BT (16, 17). Therefore, the pros and cons of frozen-thawed BT on obstetrical and neonatal outcomes remain to be investigated.

The present large-scale registry-based study revealed that FET was associated with significantly lower odds of PTB, LBW, and SGA, and higher odds of LGA. The significant reduction of <2,500 g and SGA infants could be the most beneficial outcome after FET if it were a direct result of FET, but other confounding variables can not be ruled out. These data were also consistent with earlier studies (3, 10) that showed FET to be associated with increased average birth weight of infants. For BT, the lower odds of SGA and higher odds of LGA were associated. These findings in a large population seem to be reassuring regarding neonatal safety following single frozen-thawed BT, but they must be considered in view of some studies suggesting possible abnormalities associated with blastocyst culture (18). Our data showing a possible association between increased PTB and BT is consistent with earlier papers using Swedish (4) and Canadian (15) registry data. However, the difference was not statistically significant ($P=.07$) in the present study,

TABLE 4

Odds ratios of placenta previa, placental abruption, placenta acreta, and pregnancy induced hypertension according to frozen-thawed compared to fresh and blastocyst compared to cleaved embryo among singleton pregnancies after single embryo transfer.

	Placenta previa			Placenta abruption			Placenta accreta			Pregnancy-induced hypertension		
	AOR	95% CI	*P* value	AOR	95% CI	*P* value	AOR	95% CI	*P* value	AOR	95% CI	*P* value
Maternal age (per/y)	1.05	1.02–1.08	< .001	1.04	0.99–1.10	.10	0.99	0.94–1.05	.79	1.07	1.06–1.09	< .001
Frozen-thawed/fresh	0.91	0.70–1.19	.48	0.65	0.41–1.04	.07	3.16	1.71–6.23	< .001	1.58	1.35–1.86	< .001
Blastocyst/cleaved embryo	0.90	0.69–1.19	.47	.70	0.43–1.11	.12	0.93	0.54–1.67	.78	0.94	0.80–1.10	.42
Male/female	0.93	0.76–1.15	.52	1.27	0.87–1.89	.22	0.79	0.52–1.19	.26	.91	0.81–1.02	.11

Note: Odds ratios obtained by multiple logistic regression analysis including all variables listed in the column. Abbreviations as in Table 3.

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even though more cycles were included. The significant sex ratio imbalance associated with BT was shown again with the large numbers in this study, even though this was not within the primary scope of our study, which was intended to assess the safety of FET and BT.

The most notable finding of our study was that FET was associated with higher odds of placenta accreta (AOR 3.16) than fresh ET. Although this is a very infrequent complication of pregnancy in comparison with other placenta-associated problems, it could potentially lead to hysterectomy and fatal maternal outcome. However, detailed demographics of patients having the complication of placenta accreta were not available in the registry and therefore could not be analyzed in this study. Additionally the significance of this finding remains uncertain owing to the overall limitations of the current dataset. Also, the absolute number of patients associated with increased placenta accreta was very small. However, this specific issue requires further investigation, because our study appears to be the first publication that suggests the possibility of increased placenta accreta after FET compared with fresh ET. Because we really do not even know the actual incidence of placenta accreta in spontaneously conceived pregnancies, one possible alternative hypothesis is that placenta accreta frequency is decreased in fresh ET cycles rather than increased in FET. It may be logical to consider this hypothesis if we would consider the general tendency to thicker endometrium as a result of the higher estrogenic environment in fresh cycles after ovarian stimulation.

In contrast, advanced maternal age was associated with higher odds of placenta previa, as previously described (19), but FET and BT were not. The odds of placenta abruption appeared to possibly be lower in FET, but the difference was not significant. These findings suggest that placenta-associated problems should not be discussed in isolation.

The increased PIH after FET in this paper is compatible with the results from the Swedish IVF registry (14). An earlier paper suggested increased risk of preeclampsia in cycles with BT (5), but that did not occur in this study. Because PIH has recently been suggested as a placenta-related complication during pregnancy, our study's findings merit further investigation of the peri-implantation environment, i.e., the levels of various mediators including ovarian steroids.

Because it is almost impossible to perform a large-scale prospective comparative research study in a single institution to obtain meaningful data on infrequent events related to pregnancy due to ART, meta-analysis seems to be the best possible option. However, it is obvious that the large heterogeneity of studies included in meta-analyses is a serious problem. Instead, the present study used Japanese ART registry data obtained during a relatively short period of three consecutive years from 2008 to 2010. This is currently the largest nation-wide ART registry in the world, recording ~240,000 cycles in 2010. We also extracted cycles with SET to minimize the number of variables that might affect pregnancy outcome. The very large number of cases over a short period of time could be the greatest attribute of this study. However, there are certain limitations in any type of registry-based investigation, including variable reporting and incomplete data.

The major limitation of this study is the possible wide variability of data compiled from almost 600 clinics that are different in size, location, and other characteristics. Their different protocols and criteria for the use of FET and BT potentially could bias the data they provided to the registry. Another possible limitation is the lack of a national registry of perinatal outcomes. As a result, we had to use incomplete data (90.8% of cases) on maternal and neonatal outcomes for the final analysis. However, with this high proportion of complete data, the odds results should be close to those that would be obtained with no missing data. Third, because the Japanese registry is cycle based with complete anonymity, we cannot know when oocytes were retrieved and fertilized for consecutive FET cycles. We are currently considering modifications of the registry to address this problem. In addition, it is impossible for us to know the detailed background of the patients who underwent ART, e.g., gravidity, parity, previous uterine surgery, etc. The lack of these data obviously limits the finding of an association of increased placenta accreta rate after FET, as well as the other associations.

In conclusion, this large-scale registry-based study on maternal and neonatal outcome of pregnancy after SET showed that single FET was associated with improved outcomes of neonates regarding the rates of PTB, LBW, and SGA compared with fresh transfers. Single FET did not appear to have outcomes different from fresh ET for placenta previa and placenta abruption but was associated with statistically significant higher AOR of placenta accreta and PIH. The significance of this finding requires further investigation to assess its impact on maternal safety of patients undergoing ART treatment. Single BT was associated with lower odds of SGA infants compared with cleaved embryo transfer. No association was found between single-blastocyst and single–cleaved embryo transfer for placenta previa, placenta abruption, placenta accreta, and PIH.

Even though this study is based on the largest ART registry in the world, several important limitations must be noted. First, AORs reflect association, not causality. Second, many potential confounding variables, both known and unknown, could not be considered because of the inherent limitations of registries. Third, statistical significance does not always reflect biological significance. However, with the increasing trend to single embryo transfer, the increasing application of frozen embryo transfer, and the increasing rate of blastocyst transfer, this study provides new findings and raises new questions about optimum protocols for ART patients.

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REFERENCES

1. Thurin-Kjellberg A, Olivius C, Bergh C. Cumulative live-birth rates in a trial of single-embryo or double-embryo transfer. N Engl J Med 2009;361:1812–3.
2. Grady R, Alavi N, Vale R, Khandwala M, McDonald SD. Elective single embryo transfer and perinatal outcomes: a systematic review and meta-analysis. Fertil Steril 2012;97:324–31.

3. Wikland M, Hardarson T, Hillensjo T, Westin C, Westlander G, Wood M, Wennerholm UB. Obstetric outcomes after transfer of vitrified blastocysts. Humanit Rep 2010;25:1699–707.
4. Kallen B, Finnstrom O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? Fertil Steril 2010;94:1680–3.
5. Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. Fertil Steril 2012;97:579–84.
6. Shapiro BS, Daneshmand ST, Restrepo H, Garner FC, Aquirre M, Hudson C. Matched-cohort comparison of single-embryo transfers in fresh and frozen-thawed embryo transfer cycles. Fertil Steril 2013;99:389–92.
7. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril 2012;98:368–77.
8. Roque M, Lattes K, Serra S, Sola I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril 2013;99:15662.
9. Ishihara O, Kuwahara A, Saito H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. Fertil Steril 2011;95:1966–9.
10. Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, et al. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril 2013;99:450–5.
11. Itabashi K, et al. New standard of average size and weight of newborn in Japan. Jap J Pediat 2010;114:1271–93.
12. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520–4.
13. Sullivan EA, Zegers-Hochschild F, Mansour R, Ishihara O, de Mouzon J, Nygren KG, et al. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: assisted reproductive technology 2004. Hum Reprod 2013;28:1375–90.
14. Sazanova A, Kallen K, Thurin-Kjellberg A, Wennerholm U-B, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved-thawed embryos. Hum Reprod 2012;27:1343–50.
15. Dar S, Librach CL, Gunby J, Bissonnette F, Cowan L. Increased risk of preterm birth in singleton pregnancies after blastocyst versus day 3 embryo transfer: Canadian ART Register (CARTR) analysis. Hum Reprod 2013;28:924–8.
16. Luna M, Duke M, Copperman A, Grunfeld L, Sandler B, Barritt J. Blastocyst embryo transfer is associated with a sex-ratio imbalance in favor of male offspring. Fertil Steril 2007;87:519–23.
17. Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. Fertil Steril 2009;91:2381–90.
18. Practice Committees of the ASRM and the SART. Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion. Fertil Steril 2013;99:667–72.
19. Zhang J, Savitz DA. Maternal age and placenta previa: a population-based, case-control study. Am J Obstet Gynecol 1993;168:641–5.