Increased male live-birth rates after blastocyst-stage frozen-thawed embryo transfers compared with cleavage-stage frozen-thawed embryo transfers: a SART registry study

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Objective: To investigate whether there is a difference in live-birth gender rates in blastocyst-stage frozen-thawed embryo transfers (FETs) compared with those in cleavage-stage FETs.
Design: Retrospective cohort study.
Setting: Academic medical center.
Patient(s): All women with recorded live births who underwent FET at either the blastocyst or cleavage stage, reported to the Society for Assisted Reproductive Technology during 2004–2013.
Intervention(s): None.
Main Outcome Measure(s): The primary outcome was live-birth gender rates. Demographic criteria were also collected. The chi-square analyses were used for bivariate associations, and multiple logistic regression models were used for adjusted associations, with all two-sided $P<.05$ considered statistically significant.
Result(s): A statistically significant increase was noted in the number of live male births after blastocyst-stage FET compared with that after cleavage-stage FET (51.9% vs. 50.5%). After controlling for potential confounders including age (odds ratio [OR], 1.06; 95% confidence interval [CI], 1.03, 1.08), body mass index (OR, 1.08; 95% CI, 1.04, 1.12), and male factor infertility (OR, 1.06; 95% CI, 1.03, 1.08), the increase in male live births after blastocyst-stage FET remained statistically significant.
Conclusion(s): In patients undergoing FETs, blastocyst-stage transfers are associated with higher male gender live-birth rates compared with cleavage-stage transfers. (Fertil Steril Rep® 2021;2:161–5. ©2021 by American Society for Reproductive Medicine.)
Key Words: FET, blastocyst stage, cleavage stage, gender ratio

Over the past decade, frozen-thawed embryo transfers (FETs) have become more widely used because of improved vitrification and In vitro fertilization (IVF) cycle outcomes (1). With the development of extended culture and studies reporting significant differences in outcomes between blastocyst-stage and cleavage-stage FETs, blastocyst-stage FETs are now considered the standard of care (2).

Most studies comparing cleavage-stage FET with blastocyst-stage FET have investigated embryo viability, implantation rates, live-birth rates, and pregnancy outcomes (2–4). However, minimal data have been reported regarding gender outcomes in FET cycles. Male-to-female sex ratios in fresh cycles have been reported in numerous studies suggesting an increased male live-birth rate after fresh transfer (5–13). This male-to-female sex ratio imbalance has been

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postulated to be dependent on the difference in the growth rate of embryos, with male embryos growing faster (14). Since the most advanced blastocyst is often selected for transfer, it is plausible that the sex ratio will be altered in favor of males. As more patients turn toward FET cycles, it is important to determine whether this trend toward higher male live-birth rate is also seen in FET cycles.

Using the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) database, this study aimed to investigate whether there is a difference in live-birth gender rates in blastocyst-stage FETs compared with that in cleavage-stage FETs. According to the SART, an FET is defined as a transfer occurring after the thawing of a cryopreserved oocyte or embryo. In addition, cleavage-stage transfer is defined as the transfer of a day 2–3 embryo, whereas blastocyst-stage transfer is the transfer of a day 5–6 embryo (15).

We hypothesized that blastocyst-stage FETs would have a higher male live-birth rate compared with cleavage-stage FETs. This hypothesis was based on multiple studies showing an association between fresh blastocyst-stage transfers and an increased male-to-female live-birth ratio. In addition, our hypothesis was supported by data suggesting an imbalance in gender ratio because of the increased developmental rate reported with male embryos.

MATERIALS AND METHODS

In this Institutional Review Board approved retrospective cohort study, the initial data examined were comprised of all IVF cycles reported to the SART CORS from 2004–2013. Patients who underwent fresh transfers, preimplantation genetic testing for aneuploidies, and donor cycles were then excluded. Infants who were not viable or whose gender was unknown were further excluded from the analysis. Both singleton and multiple deliveries were included. The final cohort analyzed included 98,341 live births resulting from frozen embryo transfers, at either the blastocyst stage (n = 55,867) or cleavage stage (n = 42,474).

Demographic data including body mass index (BMI), age, male factor infertility, and race/ethnicity were included in the analysis because they were considered relevant and possible confounding factors in view of recent studies suggesting that the gender ratio could be affected by such factors (11). Patient race/ethnicity was reported as follows: American Indian/Alaska Native, Asian, Black/African American, Hispanic/Latino, Mixed, Native Hawaiian/Other Pacific Islander, and White. Cases where the race/ethnicity was "not asked," "refused," or "unknown" were excluded from the adjusted analysis.

Statistical analysis was performed using both R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria) and Microsoft Excel. All P values $<.05$ were considered statistically significant. Unadjusted associations between live-birth infant gender and FET stage (blastocyst vs. cleavage) were examined using Pearson's chi-square test. Adjusted associations were examined using logistic regression models with the outcome being modeled as male infant gender (yes/no). Separate odds ratios (ORs) were calculated to control for age, BMI, race/ethnicity, and male factor infertility. This study was approved by the Rutgers Health Sciences Institutional Review Board and the SART Research Committee before the release of the data to our institution. Data were collected and verified by the SART and reported to the Centers for Disease Control and Prevention in compliance with the Fertility Clinic Success Rate and Certification Act of 1992 (Public Law 102-493). The data in the SART CORS are validated annually with some clinics having on-site visits for chart review based on an algorithm for clinic selection. During each visit, data reported by the clinic were compared with information recorded in patients' charts. Ten out of 11 data fields selected for validation were found to have discrepancy rates of $\leq 5\%$ (16).

RESULTS

Data from a total of 256,287 women who underwent FET cycles that occurred from 2004–2013 and met the study inclusion criteria were extracted. Measures from the resulting 98,341 viable infants born were analyzed. Demographic data from the FET cycles are shown in Table 1. The mean ages in the cleavage-stage and blastocyst-stage groups were 33.9 and 33.8 years, respectively. The single most frequent race/ethnicity of patients was White in the cleavage-stage (47.7%) and blastocyst-stage (44.7%) groups.

As shown in Table 2, in the cleavage-stage group, 21,470 out of a total of 42,474 (50.5%) live births were of male offspring, while in the blastocyst-stage group, 29,012 out of a total of 55,867 (51.9%) live births were male. This difference in live-birth gender rate of the blastocyst-stage group was statistically significant ($P<.001$).

Multiple logistic regression analyses were performed to individually adjust for possible confounding factors including age, BMI, ethnicity, and male factor infertility. Adjusted ORs were calculated based on these models. After controlling for age, blastocyst-stage transfer was still positively associated with a higher chance of male offspring (OR, 1.06; 95% confidence interval [CI], 1.03, 1.08). After adjusting for BMI, the increase in male live births after blastocyst-stage FET also remained statistically significant (OR, 1.08; 95% CI, 1.04, 1.12). Similarly, blastocyst-stage transfer was associated with a higher chance of a male live birth after adjusting for race/ethnicity (OR, 1.04; 95% CI, 1.01, 1.08) and male factor infertility (OR, 1.06; 95% CI, 1.03, 1.08). These results are presented in Table 3.

DISCUSSION

The altered live-birth gender ratio in favor of males resulting from fresh embryo transfers has been extensively studied. It has been suggested that this could be because of the selective transfer of male embryos. This bias likely exists at the time of embryo selection and is possibly due to a difference in the growth rate between male and female embryos (17–19). Ray et al. (18) showed that human male embryos had a higher number of cells on the second day of development and this difference continued up to the blastocyst stage compared with female embryos. Kochhar et al.'s (19) review of the mammalian literature suggests that transcripts from the

TABLE 1

Demographic characteristics.

	Cleavage-stage group		Blastocyst-stage group	
	Female infants	Male infants	Female infants	Male infants
	n = 21,004	n = 21,470	n = 26,855	n = 29,012
Mother's age				
Range	20–44	19–44	19–44	18–44
Mean (SD)	33.9 (4.1)	33.9 (4.1)	33.8 (4.1)	33.8 (4.1)
Mother's age – categorized, n (%)				
<20	0 (0.0)	2 (0.01)	1 (0.01)	2 (0.01)
20–25	376 (1.8)	405 (1.9)	541 (2.0)	604 (2.1)
26–29	2,696 (12.8)	2,796 (13.0)	3,505 (13.1)	3,770 (13.0)
30–35	10,593 (50.4)	10,617 (49.5)	13,509 (50.3)	14,452 (49.8)
36–39	5,418 (25.8)	5,740 (26.7)	6,975 (26.0)	7,630 (26.3)
>39	1,921 (9.2)	1,910 (8.9)	2,324 (8.6)	2,554 (8.8)
Ethnicity, n (%)				
American Indian/Alaska Native	33 (0.2)	25 (0.1)	29 (0.1)	34 (0.1)
Asian	1,389 (6.6)	1,447 (6.7)	2,240 (8.3)	2,417 (8.3)
Black/African American	824 (3.9)	800 (3.7)	1,022 (3.8)	1,056 (3.6)
Hispanic/Latino	833 (4.0)	838 (3.9)	1,101 (4.1)	1,217 (4.2)
Mixed	1,420 (6.8)	1,450 (6.8)	384 (1.4)	426 (1.5)
Native Hawaiian/Other Pacific Islander	31 (0.1)	44 (0.2)	57 (0.2)	50 (0.2)
White	10,011 (47.7)	10,270 (47.8)	12,103 (45.1)	12,863 (44.3)
Not asked	568 (2.7)	646 (3.0)	1,482 (5.5)	1,651 (5.7)
Refused	13 (0.1)	10 (<0.1)	18 (0.1)	15 (0.1)
Unknown	5,882 (28.0)	5,940 (27.7)	8,419 (31.3)	9,283 (32.0)
Mother's BMI				
Range	14.3–49.9	10.6–49.9	8.9–49.8	2.1–49.6
Mean (SD)	25.0 (5.3)	24.8 (5.1)	24.8 (5.2)	24.8 (5.2)
Missing/incorrectly calculated	n = 11,956	n = 12,505	n = 6,038	n = 6,778
Mother's BMI – categorized, n (%)				
Underweight (<18.5)	275 (1.3)	277 (1.3)	743 (2.8)	776 (2.7)
Normal weight (18.5–24.9)	5,280 (25.1)	5,359 (25.0)	12,293 (45.8)	13,208 (45.5)
Overweight (25–29.9)	2,090 (10.0)	2,050 (9.5)	4,689 (17.5)	5,047 (17.4)
Obese (>30)	1,403 (6.7)	1,279 (6.0)	3,092 (11.5)	3,203 (11.0)
Missing/incorrectly calculated	11,956 (56.9)	12,505 (58.2)	6,038 (22.5)	6,778 (23.4)
Father experiencing male infertility, n (%)				
No	12,854 (61.2)	13,334 (62.1)	16,272 (60.6)	17,650 (60.8)
Yes	8,150 (38.8)	8,136 (37.9)	10,583 (39.4)	11,362 (39.2)

Note: BMI = body mass index; SD = standard deviation.

Perlman. Male sex ratios frozen transfers. Fertil Steril Rep 2021.

TABLE 2

Gender ratio in blastocyst versus cleavage-stage transfer ($P < .001$).

Gender for all infants born, n (%)	Group 1: day 3 transfer n = 42,474 infants	Group 2: day 5 transfer n = 55,867 infants
Female	21,004 (49.5%)	26,855 (48.1%)
Male	21,470 (50.5%)	29,012 (51.9%)

Perlman. Male sex ratios frozen transfers. Fertil Steril Rep 2021.

TABLE 3

Odds ratios after adjusting for age, body mass index, ethnicity, and male factor infertility.

Confounder used for adjustment	Blastocyst-stage FET vs. cleavage-stage FET Odds ratio (95% CI)	*P* value
Age	1.06 (1.03, 1.08)	<.001
Ethnicity	1.04 (1.01, 1.08)	.01
BMI	1.08 (1.04, 1.12)	<.001
Male infertility factor	1.06 (1.03, 1.08)	<.001

Note: BMI = body mass index; 95% CI = 95% confidence interval; FET = frozen-thawed embryo transfer.

Perlman. Male sex ratios frozen transfers. Fertil Steril Rep 2021.

Y-chromosome function as transcription factors accelerating development, while the X-chromosome contains genes that code for rate-limiting steps in pathways key to embryo metabolism and stress reduction. In addition to differences in growth rate, gender differences in metabolic activity, which can alter oxygen radicals, have been described. Ray et al. (18) reported male embryos to have a higher metabolic activity by demonstrating a significant increase in pyruvate and glucose uptake along with lactate production compared with female embryos. Conversely, Gardner et al. (20) showed that day 4 female embryos consumed 28% more glucose compared with male embryos. Despite variable findings

reported in the literature, these studies suggest differences in male versus female embryo development and growth (20). These differences could result in biased embryo grading in favor of male embryos at the blastocyst stage.

Interestingly, most available data regarding gender outcomes are in reference to fresh cycles with minimal studies describing FET cycles. In fact, to our knowledge, there is no large nationwide study exclusively investigating the gender outcomes between blastocyst-stage and cleavage-stage FETs. A recent retrospective study by Lin et al. (21) showed that fresh IVF cycles resulted in a higher male-to-female ratio than FET cycles; however, the investigators did disclose the possibility that higher-quality embryos were more frequently transferred in the fresh cycle group. As cryopreserved embryos were the supernumerary, untransferred embryos derived from the fresh cycle, the top-quality embryos were given priority for fresh cycle transfer, suggesting that higher-quality embryos have a priority for fresh transfer and that male embryos are selected more predominantly for fresh cycle transfer. Similarly, Supramaniam et al. (13) revealed a significantly skewed secondary sex ratio in favor of male live offspring in the case of all IVF treatment cycles. Further analysis showed an increased male-to-female ratio in fresh blastocyst-stage transfer compared with that in fresh cleavage-stage transfer regardless of the use of intracytoplasmic sperm injection (ICSI) or conventional IVF. However, when stratifying for FET cycles, the group found no statistically significant difference in sex ratio. Multiple other studies deriving data from national databases have supported increased male live births after fresh cycles (5, 7, 11, 22). In contrast, the results of other studies have shown no differences in the male-to-female ratio after fresh IVF cycles, but these studies are in the minority (23, 24).

Our results show that in accordance with the results from multiple studies in fresh cycles, the male-to-female live-birth ratio appears to be significantly associated with the blastocyst-stage transfer in FET cycles. Although statistically significant differences were noted in live-birth gender, differences may be because of our large sample size and may not be clinically meaningful. Despite the small difference, when adjusted for potential confounding factors such as maternal age, race/ethnicity, male factor infertility, and BMI, this association remained significant. The predictors included in the models were selected based on prior reports showing a potential association with sex ratio (11). Our data set failed to include the use of ICSI, which has been linked to lower proportions of male live births (7, 22). As a surrogate for this missing information, male factor infertility was used as a variable in our analysis.

These results highlight the fact that inadvertent biased sex selection is a very real possibility in FET as well as fresh IVF cycles, especially in the case of blastocyst transfer. It would be important to include this possible bias in the counseling of patients considering IVF because they may want to consider a random selection of the embryo to be transferred.

The main strength of this study is the large number of cases reported nationwide and the ability to perform subgroup analysis. One of the limitations, besides being a retrospective study, is the discrepancy in clinical reporting. In this data set, both race/ethnicity and BMI were not well-populated fields. Another limitation of this data set was the inability to determine which FETs are of supernumerary embryos compared with embryos generated from a freeze-all cycle. Despite the significance of our results, it is important to mention that our analysis did not include stratification for the use of ICSI, number of embryos transferred, or preimplantation genetic testing for aneuploidies. In our data set, the few number of cycles recorded as having undergone preimplantation genetic testing for aneuploidies precluded us from performing meaningful analysis, and these were excluded from the data set. Additionally, one must be aware of the differences in practice patterns and changes in the laboratory that may have occurred compared with when these data were collected. As seen in our data (Table 4), over time, a shift from cleavage-stage FET to blastocyst-stage FET has become the standard of care.

In addition, it is important to recognize that a skewed sex ratio in favor of male live birth occurs in natural conception as well. This imbalance has been extensively studied and has been found to be associated with multiple factors including

TABLE 4

Cleavage-stage versus blastocyst-stage transfer per year.

Year	Cleavage-stage FET	Total resulting live births with known gender	% males among live births	Blastocyst-stage FET	Total resulting live births with known gender	% males among live births
2004	4,272	5,323	51.1	0	NA	NA
2005	4,797	5,946	50.9	0	NA	NA
2006	4,412	5,473	50.9	804	994	53.6
2007	3,678	4,565	50.3	2,106	2,630	52.7
2008	3,245	3,996	49.3	3,260	4,009	53.2
2009	2,780	3,444	50.3	4,203	5,152	51.0
2010	2,803	3,500	50.1	5,561	6,869	51.4
2011	2,864	3,462	50.8	6,887	8,501	52.5
2012	2,762	3,286	49.9	9,739	11,950	52.4
2013	2,943	3,479	51.2	13,061	15,762	51.3
Total	34,556	42,474	50.5	45,621	55,867	51.9

Note: FET = frozen-thawed embryo transfer; NA = not available.

Perlman. Male sex ratios frozen transfers. Fertil Steril Rep 2021.

maternal age, paternal age, maternal stress, race, and parental hormone levels at the time of conception (25). Sex ratios at birth from natural conception could be used to compare with assisted reproductive technology outcomes in future studies.

Finally, given the underlying hypothesis that the skewed sex ratio at birth is because male embryos have a faster growth rate, it is important to consider whether this difference is reflective of a difference in viability and/or implantation potential. If the aforementioned were to be true, then it would be reasonable to postulate that the sex ratio would be unchanged regardless of selection bias at the time of transfer. It would be important to address the aforementioned questions in future studies.

CONCLUSION

Blastocyst-stage FET results in higher male live-birth rates compared with cleavage-stage FET. These findings support previously reported data regarding fresh transfers in terms of live-birth sex ratio differences. Our data support previous suggestions that embryo grading systems prioritize male embryos for transfer, leading to a sex ratio imbalance.

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