Influence of embryo sex on development to the blastocyst stage and euploidy

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Objective: To compare the prevalence of blastocyst development and euploidy in XX versus XY embryos.
Design: Retrospective cohort study.
Setting: Boston IVF, a large university-affiliated reproductive medicine practice.
Patient(s): All patients who underwent their first preimplantation genetic screening cycle between January 1, 2006, and December 31, 2007.
Intervention(s): In vitro fertilization and preimplantation genetic screening.
Main Outcome Measure(s): Proportion of embryos that developed to the blastocyst stage by day 5 and prevalence of euploidy for chromosomes 8, 13, 14, 15, 16, 17, 18, 20, 21, and 22 in XX versus XY embryos.
Result(s): Seven hundred fifty-eight embryos from 138 cycles in 138 patients were analyzed. Three hundred sixty-six (48%) were XX, and 392 (52%) were XY. XX and XY embryos were equally likely to develop to the blastocyst stage by day 5 and were equally likely to be euploid for the analyzed chromosomes.
Conclusion(s): Our data suggest that extending embryo culture to day 5 does not lead to sex selection and that euploidy and aneuploidy are not sex dependent. (Fertil Steril® 2011;95:936–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Preimplantation genetic screening, euploidy, aneuploidy, blastocyst, in vitro fertilization, assisted reproductive technology, ART outcomes

The development of sequential culture media sparked considerable interest in the possibility of improving IVF outcomes by extending embryo culture to the blastocyst stage. Since the introduction of G1/G2 media by Gardner et al. (1) in the 1990s, increasing evidence has emerged in support of improved implantation rates, pregnancy rates, and live birth rates with blastocyst transfer compared with day 3 transfer (2–4). In select patients, transferring fewer embryos on day 5 yields decreased multiple gestation rates while maintaining comparable pregnancy rates (5).

Potential theories in support of blastocyst transfer include improved synchronicity between the uterine environment and the embryo, as well as improved embryo selection. The vast majority of day 3 embryos are aneuploid, and morphology-based embryo selection is highly imperfect (6). Embryos that become day 5 blastocysts are reportedly more likely to be euploid; however, the prevalence of aneuploidy is still relatively high (7, 8).

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Despite the evidence for improved outcomes with blastocyst transfer, potential concerns include a higher risk of cancelled cycles (9), fewer embryos available for cryopreservation (10), an increased monozygotic twinning risk (11, 12), and an altered sex ratio (13). One possible mechanism for an altered sex ratio is an increased developmental rate in male embryos. Multiple authors have demonstrated increased cellularity and shorter time to blastocoele formation in murine (14–16), bovine (17–19), ovine (20), and porcine (21) male embryos compared with female embryos. Proposed mechanisms include metabolic differences (22), Y-linked gene expression (15, 23, 24), and epigenetic effects (25). Recent studies also have reported that human male cleavage stage embryos and blastocysts have an increased number of cells compared with female embryos, raising concern for the possibility that blastocyst transfer may select for faster-developing male embryos (26, 27). Currently, the data are mixed regarding the impact of blastocyst transfer on sex ratio. Existing studies demonstrating a skewed sex ratio have drawn their conclusions from the sex ratio of liveborn infants (28–32). This primary outcome is only an indirect measure of embryo developmental rates, because it does not account for potential confounders such as differences in implantation and loss rates between female and male embryos. The only existing study in which blastocyst sex was controlled for did not demonstrate an alteration of the sex ratio (33).

In light of the conflicting data regarding sex ratios after blastocyst transfer and the evidence in support of increased developmental rates in male embryos, we aimed to examine specifically the likelihood of development to the blastocyst stage by day 5 in human XX versus XY embryos. We hypothesized that male embryos would be more likely to become blastocysts by day 5 than female embryos. Given that day 5 blastocysts have a higher prevalence of euploidy

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TABLE 1

Baseline patient characteristics and indications for preimplantation genetic screening.

Characteristic	Value
Age (y)	37.6 ± 4.0
Day 3 FSH (mIU/mL)	7.6 ± 2.4
Gravidity	2.0 ± 1.7
Parity	0.6 ± 0.8
Cycle no.	2.9 ± 2.1
Indication for preimplantation genetic screening	
Advanced maternal age, no. (%)	67 (49)
Recurrent miscarriage, no. (%)	29 (21)
Recurrent IVF failure, no. (%)	42 (30)

Note: Values are in mean ± SD or number (%).

Eaton. Embryo sex and blastocyst development. Fertil Steril 2011.

TABLE 2

Euploidy prevalence by tested chromosome among day 3 embryos that became day 5 blastocysts.

Chromosome	XX (%)	XY (%)	*P* value
8	96/106 (91)	98/113 (87)	.37
13	168/212 (79)	187/223 (84)	.17
14	72/99 (73)	82/102 (80)	.12
15	169/203 (83)	170/212 (80)	.39
16	160/205 (78)	186/218 (85)	.04
17	173/193 (90)	188/207 (91)	.68
18	176/211 (83)	186/224 (83)	.90
20	72/93 (77)	77/100 (77)	.93
21	173/213 (81)	184/226 (81)	.96
22	165/203 (81)	179/218 (82)	.82

Note: Denominator = number of embryos with a result for the chromosome of interest.

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than day 3 embryos, we also hypothesized that male embryos would be more likely to be euploid than female embryos. Therefore, a secondary goal of our study was to assess the prevalence of euploidy in XX versus XY embryos.

MATERIALS AND METHODS

Subjects

The Committee on Clinical Investigations at Beth Israel Deaconess Medical Center approved this study. We retrospectively identified all women who underwent IVF with their first preimplantation genetic screening cycle for advanced maternal age, recurrent miscarriage, or recurrent IVF failure from January 1, 2006, through December 31, 2007, at Boston IVF. Women who had received prior IVF treatment at facilities other than Boston IVF were excluded from the analysis.

Protocols

Patients underwent ovarian stimulation protocols with gonadotropins and either a GnRH agonist or antagonist as previously described (6). Cycles were monitored with serum E_2 levels and transvaginal ultrasound examinations beginning on treatment days 6 to 8. When at least three follicles measured 15 to 20 mm, either 250 μg recombinant hCG (Ovidrel; EMD Serono) or 10,000 U of urinary hCG (Novarel; Ferring Pharmaceuticals) was administered SC. Ultrasound-guided oocyte retrieval was performed 36 hours after hCG administration.

Assessment of embryo morphology and biopsy of a single blastomere from day 3 embryos with at least four cells were performed as previously described (6). The fixed cells were sent to Reprogenetics (Livingston, NJ) for fluorescence in situ hybridization analysis. If centromeric probes were not sufficient, telomeric probes were used (34). Before July 2006, embryos were analyzed with a nine-chromosome panel including chromosomes X, Y, 13, 15, 16, 17, 18, 21, and 22. Beginning July 1, 2006, embryos were analyzed either with the nine-chromosome panel or with a 12-chromosome panel including the nine-chromosome panel plus chromosomes 8, 14, and 20. Only blastocysts without evidence of aneuploidy were transferred on day 5. The number of embryos transferred was based on nationally published guidelines and patient-specific data. Euploidy was defined as two copies of the chromosome of interest in the case of autosomes and two sex chromosomes.

Outcomes

The primary outcome was the proportion of XX versus XY embryos that became day 5 blastocysts. A secondary outcome was the prevalence of euploidy for each analyzed autosome in XX versus XY embryos. All XX and XY embryos with a result for the autosome of interest were included in the individual chromosomal euploidy analyses. Embryos with no report for a particular tested chromosome were excluded from analysis of euploidy for that chromosome.

Statistical Analysis

All analyses were conducted with use of the Statistical Analysis System (SAS 9.2; SAS Institute, Cary, NC). Categorical data was analyzed with the χ^2 test. Proportions of euploidy in the XX and XY groups were compared with use of repeated-measures logistic regression to adjust *P* values to account for lack of independence among embryos from the same woman. A *P* value of $<.05$ was considered statistically significant.

RESULTS

Seven hundred fifty-eight embryos from 138 cycles in 138 patients were analyzed. Baseline patient characteristics and indications for preimplantation genetic screening are listed in Table 1. Three hundred sixty-six embryos (48%) were XX, and 392 (52%) were XY. XX and XY embryos were equally likely to develop to the blastocyst stage by day 5 (58% and 58%, respectively, $P=.88$). Of the 439 embryos that became day 5 blastocysts, XX and XY embryos were equally likely to be euploid for individual autosomes except for a higher proportion of euploidy for chromosome 16 among male embryos (Table 2).

Three hundred ten embryos had complete results for the 12-chromosome panel. Among those embryos, 143 (46%) were XX and 167 (54%) were XY. As shown in Table 3, the prevalence of euploidy for the analyzed chromosomes was similar for XX and XY embryos ($P=.96$). Among embryos with complete results for the 12-chromosome panel, 187 (60%) developed to the blastocyst stage by day 5 and 123 (40%) did not. The prevalence of euploidy for the analyzed chromosomes was similar for XX and XY embryos that became day 5 blastocysts ($P=.70$), as well as for those that did not ($P=.79$).

DISCUSSION

Our data suggest that XX and XY embryos are equally likely to develop to the blastocyst stage by day 5 and equally likely to be euploid. On the basis of a review of the published literature, we believe that our study is the first to examine specifically the relationship between human embryo sex and development to the blastocyst stage, as well as the prevalence of euploidy for individual autosomes among XX and XY embryos. Given the controversy regarding

TABLE 3

Prevalence of euploidy for the complete 12-chromosome panel.

	XX (%)	XY (%)	*P* value
All embryos (N = 310)	32/143 (22)	37/167 (22)	.96
Day 5 blastocysts (N = 187)	25/90 (28)	29/97 (30)	.70
Nonblastocysts (N = 123)	7/53 (13)	8/70 (11)	.79

Eaton. Embryo sex and blastocyst development. Fertil Steril 2011.

blastocyst transfer and sex ratios, our findings are clinically significant because they suggest that extending embryo culture to day 5 does not select for male embryos. The importance of the finding is relevant to any patient wishing to undergo preimplantation genetic screening or preimplantation genetic diagnosis because they all must have an ET at the blastocyst stage.

Although the animal data supporting increased developmental rates in male embryos are strong (14–21), human data are limited. Tarin et al. (35) calculated the difference in mean cell number between transferred embryos and nontransferred embryos. The authors found that when this difference was greater than zero, the sex ratio was skewed toward males. In their study, however, ET was performed on day 2 and therefore not representative of outcomes for blastocyst transfer. Moreover, they did not determine or control for the sex of the preimplantation embryos. Ray et al. (27) reported a statistically significantly increased number of cells in male embryos on day 2; however, statistical significance was not maintained on day 3 or 6. Additionally, their analysis included only discarded embryos; thus, their findings may lack clinical significance. A similar analysis by Dumoulin et al. (26) demonstrated an increased mean log cell number in male blastocysts after intracytoplasmic sperm injection but not IVF.

The impact of blastocyst transfer on offspring sex ratio is equally poorly understood. There are few studies demonstrating that blastocyst transfer alters the sex ratio, and no studies specifically demonstrate a causal relationship between embryo development and sex ratio after blastocyst transfer. A recent meta-analysis that included four retrospective studies demonstrated a higher male-female ratio after blastocyst transfer compared with cleavage-stage ET (13). One of the studies included in the meta-analysis demonstrated that the sex ratio was shifted in favor of males after blastocyst transfer; however, male and female blastocysts had similar morphologic grades based on the degree of blastocoele expansion and cellularity of the inner cell mass and trophectoderm (28). This finding suggests that an altered sex ratio is unlikely to be a result of preferential selection of male embryos based on morphology. Another included study demonstrated a trend toward an increased male-to-female ratio after blastocyst transfer compared with day 3 transfer, but statistical significance was achieved only after their data were pooled with data from the literature (29). Menezo et al. (30) reported that blastocyst transfer skewed the sex ratio toward males; however, their control population consisted of infants delivered after spontaneous conception rather than day 3 transfer. A small study assessing sex ratio after blastocyst transfer in 10 women found that three quarters of the infants were males, but there was no comparison group (31). None of these studies accounted for the sex of the blastocyst; thus, it is unclear whether the imbalanced sex ratio at birth is due to a higher proportion of male blastocysts or a process occurring after ET that favors male embryos.

Several studies have failed to demonstrate an altered sex ratio at birth after blastocyst transfer (36–38). Recently, Richter et al. (39) used logistic regression analysis to assess the relationship between selection for embryonic developmental rate and offspring sex. The percentage of male births was approximately 50%, regardless of the degree of selection for developmental rate. The only study that controlled for the sex of the blastocyst found no difference in the male-to-female delivery ratio per embryo transferred (33). Only blastocyst transfers were included in the analysis; however, an assessment of day 3 morphology revealed a similar grade and mean cell number for female and male embryos, indicating similar developmental rates.

Our study provides further evidence in support of prior studies that failed to demonstrate a difference in developmental rates between female and male human embryos. The data suggest that the offspring sex ratio is not likely to be altered by developmental rate and that alternative causes such as differential implantation or loss rates should be considered. Furthermore, any differences in implantation or loss rates are not likely to be a result of aneuploidy, given that male and female embryos were equally likely to be euploid for all individually analyzed autosomes in our study except for chromosome 16. In our high-risk population of patients undergoing preimplantation genetic screening, a significant proportion of both male and female embryos that became day 5 blastocysts and had complete results for the twelve-chromosome panel were aneuploid. A similar proportion of aneuploid embryos from comparable patient populations has been demonstrated in the existing literature (40, 41).

Limitations of our study include the typical limitations of preimplantation genetic screening, notably false-positive and false-negative results with fluorescence in situ hybridization and the potential for mosaicism that would not be detected with biopsy of only one blastomere. Additionally, because only a relatively small number of patients having preimplantation genetic screening with a high risk of aneuploidy were included, it is unclear whether our findings can be extrapolated to patients with a lower risk of aneuploidy.

Patients should be counseled that the data regarding blastocyst transfer and sex ratios are limited and that the relationship between blastocyst transfer and offspring sex ratios is poorly understood. Our data suggest that female and male embryos are equally likely to develop to the blastocyst stage by day 5 and those that become day 5 blastocysts are equally likely to be euploid for chromosomes tested by preimplantation genetic screening on day 3. Further research is needed to elucidate the relationships among blastocyst development, embryo sex, and euploidy.

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