Influence of patient age on the association between euploidy and day-3 embryo morphology

A retrospective cohort study conducted in 138 patients undergoing their first preimplantation genetic screening (PGS) cycle between January 1, 2006, and December 31, 2007, demonstrated that embryos with good day-3 morphology were more likely to be euploid for X/Y, 8, 15, and 18 than those with poor morphology. The strength of association between euploidy and day-3 morphology was not influenced by maternal age. (Fertil Steril® 2010; 94:365–7. ©2010 by American Society for Reproductive Medicine.)

Day-3 embryo morphology is the most commonly used method to select embryos for transfer (1); however, it does not definitively correlate with euploidy (2). Many morphologically normal embryos that become day-5 blastocysts are aneuploid, particularly in women at high risk for aneuploidy (3). Because aneuploid embryos may appear morphologically normal and develop to the blastocyst stage, preimplantation genetic screening (PGS) increasingly has been used to improve embryo selection in certain patient populations. Although existing randomized, controlled trials have not demonstrated improved pregnancy rates with PGS (4–6), there are data to suggest that PGS may improve detection of specific chromosomal abnormalities that are compatible with development to the blastocyst stage, implantation, and subsequent development to term (7).

The risk of aneuploidy increases with advancing maternal age (2), and the average maternal age continues to rise in the United States (8). Studies also have shown decreased cleavage rates and increased fragmentation in embryos from older patients (9). It is not known whether age impacts the usefulness of morphology as an embryo selection tool. Therefore, our study assessed the influence of maternal age on the relationship between euploidy and embryo morphology.

The study was approved by the institutional review board at Beth Israel Deaconess Medical Center. We retrospectively identified all women who underwent in vitro fertilization (IVF) with their first PGS cycle for advanced maternal age, recurrent miscarriage, or recurrent IVF failure between January 1, 2006, and December 31, 2007, at Boston IVF. Women with other indications for PGS and those who had undergone prior IVF cycles at facilities other than Boston IVF were excluded.

Patients underwent ovarian stimulation protocols with gonadotropins and either a gonadotropin-releasing hormone (GnRH) agonist or antagonist, as previously described elsewhere (7). Cycles were monitored with serum estradiol levels and transvaginal ultrasounds beginning on treatment days 6 to 8. When at least three follicles measured 15 to 20 mm, either 250 μg of recombinant human chorionic gonadotropin (hCG; Ovidrel; EMD Serono, Rockland, MA) or 10,000 units of urinary hCG (Novarel; Ferring Pharmaceuticals, Parsippany, NJ) were administered subcutaneously. Ultrasound-guided oocyte retrieval was performed 36 hours after hCG administration.

Assessment of embryo morphology was performed daily beginning 1 day after oocyte retrieval. Embryos were designated as having high implantation potential (HIP) if they had at least 4 cells on day 2, and 7 to 10 cells on day 3, with $<$20% fragmentation and no multinucleation. Biopsy of a single blastomere from day-3 embryos with at least 4 cells was performed using either a laser (Zilos; Hamilton Thorne Biosciences, Beverly, MA) or acidified Tyrode's (SAGE; Cooper Surgical, Trumbull, CT) to breach the zona. Nuclear fixation was performed using a modified Carnoid method (10). The fixed cells were sent to Reprogenetics (Livingston, NJ) for fluorescence in situ hybridization. If centromeric probes were not sufficient, telomeric probes were used. 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 twelve-chromosome panel that included the nine-chromosome panel plus chromosomes 8, 14, and 20.

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TABLE 1

Association between euploidy and embryo morphology stratified by maternal age.

Chromosome	Maternal age in years adjusted risk ratio[a] (95% confidence interval)				Test for interaction *P* value
	<35	35 to <38	38 to <41	≥41	
XY	1.44 (0.86–2.42)	1.71 (0.68–4.35)	1.19 (0.88–1.61)	1.45 (0.51–4.13)	.57
8	1.28 (0.79–2.08)	1.91 (0.86–4.29)	1.15 (0.99–1.35)	1.41 (0.62–3.24)	.68
13	1.53 (0.78–3.03)	1.23 (0.92–1.65)	1.03 (0.75–1.40)	0.99 (0.59–1.67)	.24
14	1.71 (1.01–2.90)	1.15 (0.84–1.57)	1.07 (0.78–1.49)	1.18 (0.62–2.22)	.13
15	1.25 (0.79–2.00)	1.27 (0.91–1.78)	0.98 (0.78–1.24)	1.53 (0.98–2.39)	.94
16	1.26 (0.82–1.94)	1.11 (0.81–1.52)	1.16 (0.82–1.64)	0.96 (0.65–1.42)	.48
17	1.36 (0.74–2.49)	1.39 (0.81–2.37)	0.86 (0.67–1.10)	0.88 (0.49–1.56)	.16
18	1.48 (0.92–2.39)	1.34 (0.97–1.86)	1.15 (0.82–1.62)	0.95 (0.60–1.51)	.17
20	0.84 (0.51–1.39)	1.05 (0.69–1.60)	1.06 (0.83–1.36)	0.95 (0.59–1.54)	.68
21	1.49 (0.94–2.37)	1.20 (0.89–1.63)	0.84 (0.66–1.08)	0.92 (0.55–1.54)	.06
22	1.48 (0.83–2.67)	1.45 (1.05–1.99)	0.98 (0.75–1.27)	0.87 (0.62–1.23)	.06

[a] Adjusted for maternal age as a continuous variable.

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The main outcome of interest was the influence of maternal age on the association between euploidy and embryo morphology, as defined by HIP status, for each individually analyzed chromosome. Euploidy was defined as two copies of the autosome of interest. For the chromosomes involved in sex determination, euploidy was defined as XX or XY. All embryos with a result for the chromosome of interest were included in the individual chromosomal euploidy analyses. Embryos that were nonreactive for a particular chromosome were excluded from analysis of euploidy for that chromosome.

All analyses were conducted using the Statistical Analysis System (SAS 9.1.3; SAS Institute, Cary, North Carolina). Repeated measures log-binomial regression, which accounted for the lack of independence among embryos from the same woman, was used to generate risk ratios (RR) and 95% confidence intervals (CI). The risk ratios for each stratum of maternal age also were adjusted for continuous maternal age. The test for interaction was used to demonstrate whether the RR for each chromosome varied across age strata. $P<.05$ was considered statistically significant.

Our computerized IVF database identified 138 patients who underwent their first PGS cycle. A total of 1048 embryos were analyzed by PGS. Of these, 521 became day-5 blastocysts. These 521 embryos came from 123 cycles in 123 patients. Patient age was <35 years for 111 embryos, 35 to <38 years for 165 embryos, 38 to <41 years for 138 embryos, and ≥41 years for 107 embryos. The indication for PGS was advanced maternal age in 60 patients, recurrent miscarriage in 26 patients, and recurrent IVF failure in 37 patients.

The HIP embryos that developed to the blastocyst stage by day 5 were statistically significantly more likely than non-HIP embryos to be euploid for chromosomes X/Y (90% vs. 82%, $P=.03$), 8 (86% vs. 76%, $P=.04$), 15 (81% vs. 71%, $P=.02$), and 18 (82% vs. 73%, $P=.02$). Among the remaining chromosomes, there was no statistically significant association between euploidy and HIP status.

Table 1 shows the adjusted risk ratios for the association between euploidy and morphology stratified by maternal age. The test for interaction did not demonstrate a statistically significant influence of maternal age. Complete results for the nine-chromosome panel were available for 438 embryos. Again, there was no statistically significant influence of maternal age on the association between euploidy and day-3 morphology ($P=.32$): <35 years: RR 1.34 (95% CI, 0.97–1.85); 35 to < 38 years: RR 1.20 (95% CI, 0.97–1.49); 38 to <41 years: RR 1.14 (0.88–1.47); ≥ 41 years: RR 1.00 (95% CI, 0.59–1.68).

To our knowledge, this is the first study to analyze the influence of maternal age on the relationship between euploidy and day-3 embryo morphology. We found that maternal age did not statistically significantly impact this relationship.

We previously demonstrated that day-3 morphology does not correlate with euploidy for chromosomes 13, 20, or 21 in embryos that become day-5 blastocysts (7). Therefore, one potential role for PGS may be the detection of chromosomal defects that are compatible with development to the blastocyst stage, implantation, and ultimately a term infant. Our study showed a statistically significant correlation between HIP morphology and euploidy for chromosomes X/Y, 8, 15, and 18. There was no statistically significant relationship between HIP morphology and euploidy for the remaining chromosomes. Therefore, this study supports our previous findings.

Our study is limited by the relatively small number of embryos within each age group. Reanalyzing the data in the future with larger numbers of embryos will reduce the possibility of type II error.

Day-3 embryo morphology correlates with euploidy for some, but not all, chromosomes. Our data suggest that maternal age does not impact the relationship between euploidy and day-3 morphology. Day-3 morphology remains an imperfect method for selection of euploid embryos from younger and older patients alike.

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