

Intra-age, intercenter, and intercycle differences in chromosome abnormalities in oocytes

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Objective: To determine the extent of intra-age and intercycle variations in the frequency of first polar body aneuploidy in two consecutive cycles of oocyte retrieval undertaken by the same patient within 1 year.

Design: Retrospective study.

Setting: Fertility centers.

Patient(s): Infertile couples undergoing IVF.

Intervention(s): Patients underwent two consecutive cycles of preimplantation genetic screening through first polar body biopsy within 1 year.

Main Outcome Measure(s): Meiosis I aneuploidy.

Result(s): A total of 226 patients underwent 452 cycles of preimplantation genetic screening. Differences within age groups were wide, with 0–100% of oocytes being chromosomally normal in all age groups. Euploidy rates between centers were significantly different (48% vs. 25%). Intercycle differences for the same patient were also wide (0–100%), but with 68.5% of patients having less than ± 2 euploid eggs of difference between cycles.

Conclusion(s): Although euploidy rate decreased on average with advancing maternal age, the high intra-age and intercenter variation in oocyte chromosome abnormalities emphasize the difficulty in estimating how many euploid oocytes a specific woman will have. This may have repercussions for fertility preservation where a defined number of eggs are currently frozen just based on maternal age. (*Fertil Steril*® 2012;97:935–42. ©2012 by American Society for Reproductive Medicine.)

Key Words: PGD, preimplantation genetic diagnosis, aneuploidy, cryopreservation, fertility preservation, cancer, PGS

Fertility decreases with advancing maternal age, with implantation rates declining from 30% in women younger than 35 years to 6% in women 41–42 years old (1). This is primarily due to an increase in chromosome abnormalities in the oocytes as a result of aging (2, 3), and can be reversed by oocyte donation from younger to older women (4). Also, preliminary data have shown that preimplantation genetic screening (PGS), with full chromosome analysis using microarrays coupled with blastocyst biopsy, ameliorates the decrease in implantation rates with

advancing maternal age when euploid embryos are identified and replaced (5–8).

Polar body (PB) analysis is less commonly undertaken for PGS because it requires more samples (i.e., the first and second PBs) from eggs that may or may not reach blastocyst stage. However, in some countries such as Germany and Italy, legal restrictions do not allow PGS after fertilization of an oocyte and render PBs the only cells that can be analyzed.

Great strides have been made in oocyte freezing in the past few years, especially after the advent of vitrification

(9). Oocyte freezing is now quite successful (10), having already produced similar pregnancy rates (PR) to those obtained in unfrozen, fresh oocyte cycles (11–14). This has allowed many advances in the field of assisted reproduction, including better coordination of egg donor cycles through egg banking (11, 13), fertility preservation (FP) for women diagnosed with cancer (12, 15, 16), and FP for women who wish to delay childbearing (17, 18).

A survey on oocyte cryopreservation revealed that 51% of infertility clinics in the United States offered this service, and 66% of women who wanted to freeze their oocytes intended to delay childbearing for social reasons, 18% because of cancer, and the remainder for other reasons (18). Fifty percent of the US clinics providing oocyte cryopreservation offered this service exclusively to women up to 40 years of age for elective purposes (18). This illustrates that egg freezing is being offered increasingly to women 35

Received June 25, 2011; revised January 11, 2012; accepted January 17, 2012; published online February 11, 2012.

S.M. reports stock/stock options with Reprogenetics. K.R.H. has nothing to disclose. C.M.M. has nothing to disclose. B.A. has nothing to disclose. D.W. reports that he is employed by and has stocks/stock options with Reprogenetics. E.F. reports that she is employed by Reprogenetics. V.B. has nothing to disclose. R.F. has nothing to disclose. L.G. has nothing to disclose.

Supported by National Institute for Health Research (NIHR) Biomedical Research Centre Programme (D.W.).

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Fertility and Sterility® Vol. 97, No. 4, April 2012 0015-0282/\$36.00

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doi:10.1016/j.fertnstert.2012.01.106

years and older, who have a higher risk of producing chromosomally abnormal oocytes. Elective oocyte cryopreservation as a preventative approach would ideally be offered before the age of 35 years; however, most women undergoing social egg freezing are of advanced reproductive age, most likely as a result of career and economic reasons. It is usually suggested to cryopreserve numerous oocytes to counterbalance the anticipated decrease in oocyte quality observed in women of advanced reproductive age. However, there is no empirical data available concerning the ideal number of oocytes that should be frozen to preserve fertility.

Furthermore, small studies involving egg donors (19), women who underwent two PGD cycles (20), and unpublished data from our centers on more than 20,000 PGD procedures indicate great variation in aneuploidy rates between women of the same age, and even between consecutive cycles for the same patient. All this evidence comes from day 3 biopsies and thus postmeiotic abnormalities, paternal contribution or differences in culture conditions, and hormonal stimulation between cycles (21) may have contributed to those observations.

The purpose of this study is to evaluate intra-age as well as intercycle variations in the incidence of euploid oocytes per PGS cycle in a large cohort of oocytes from women who underwent two consecutive PGS procedures by PB analysis.

MATERIALS AND METHODS

Infertile couples that underwent two consecutive IVF/intracytoplasmic sperm injection (ICSI) cycles with PGS within 1 year at Fertility Center of Hamburg, Hamburg, Germany, or at SISMeR, Bologna, Italy, were included in the present study. Patients consented to PGS but for the rest of the study it was determined to be exempt from institutional review board approval. Patients recruited in both centers underwent PGS for the indications of advanced maternal age, recurrent pregnancy loss, or repeated implantation failure.

According to the Western Institutional Review Board in Olympia, Washington, under common rule 45 CFR 46.101(b) (4), exemptions include “research, involving the collection or study of existing data, documents, records, pathological specimens, if these sources are publicly available or if the information is recorded by the investigator in such manner that subjects cannot be identified, directly or through identifiers linked to subjects.”

Fluorescence in Situ Hybridization PB Procedures Performed in Italy

Ovarian stimulation was performed by administering exogenous gonadotropins after a long desensitization protocol with long-acting GnRH analogues (22, 23). Oocytes were collected transvaginally by ultrasound guidance 36 hours after hCG administration, and cultured in human tubal fluid (HTF) (Quinn's Advantage Fertilization HTF medium; SAGE CooperSurgical Inc.) supplemented with 5% human serum albumin (HAS; SAGE) at 37°C in a 5% CO₂ humidified gas atmosphere.

The PB biopsies in the Bologna center were performed approximately 1 hour after oocyte retrieval using mechanical biopsy by using a micromanipulator with a double holder that carried a partial zona dissection glass microneedle and a PB biopsy capillary. Because of Italian law, not all oocytes were fertilized, and only those to be fertilized were biopsied.

A slit of approximately 20 μm was made mechanically in the zona pellucida (ZP) by passing the partial zona dissection microneedle through the perivitelline space tangentially to the oocyte, and the cut completed by repeatedly rubbing the microneedle against the holding capillary (24). When the slit was opened, the PBs were gently aspirated with the biopsy capillary. Fixation of polar bodies onto SuperFrost glass slides (Menzel-Gläser) was performed using four cycles with three parts methanol and one part acetic acid and subsequent one cycle with pure methanol, followed by fluorescence in situ hybridization (FISH) with six probes specific for chromosomes 13, 15, 16, 18, 21, and 22 (Multivision PB Panel, CEP 15 Spectrum Orange; Abbott Laboratories). Hybridization was carried out on the fixed PBs for a minimum of 2 hours. After counterstaining in antifade solution (Antifade II; Abbott) slides were scored by two independent observers at a fluorescence microscope (Olympus BX41; Olympus) and images were captured at ×600 magnification using a CCD PVCAM camera associated with image analysis software (Vysis Quips). Insemination was performed by ICSI on the basis of FISH results by introducing the injection needle through the breach already opened in the ZP (25).

Reanalysis was performed on 43 oocytes that were not clinically used by testing the same chromosomes with the same probes used for PB analysis. The error rate was calculated by comparing the result of the PB analysis with that of the oocyte. A missing or extra specific chromosome (i.e., nullisomy 21 or disomy 21) in the PB should result in an extra or missing same chromosome in the oocyte (i.e., disomy 21 or nullisomy 21), respectively. Any other combination was considered an error.

FISH PB Procedures Performed in Germany

All patients were stimulated with the long GnRH agonist protocol. Women received oral contraceptive (OC) pills for 14–21 days starting from the first day of the preceding menstrual cycle. Daily 0.8 mg nafarelin (Synarela, Pharmacia) was administered intranasally starting from the last 7 days of OC pill use. Gonadotropin injections were started after menstrual bleeding when the women had received at least 10 days of GnRH agonist. Pituitary suppression was confirmed with the absence of ovarian cysts, serum E₂ level <50 pg/mL, and P level <1 ng/mL. Recombinant FSH and LH was used in a 2:1 ratio (Pergoveris, MerckSerono) for ovarian stimulation. Daily gonadotropin dosage ranged between 150:75 IU and 300:150 IU depending on anticipated ovarian response. Final oocyte maturation was triggered with 250 μg of recombinant hCG (Ovitrelle, MerckSerono) when at least three follicles were ≥ 18 mm.

The method of PB analysis has been described in detail previously (26, 27). First and second PBs were extracted simultaneously from normally fertilized oocytes (those with

presence of two pronuclei [2PN]) about 12 (ICSI) to 14 hours (IVF) after insemination after breaching the ZP by thermoablation using a laser system (Fertilase, with Octax Eye Wear system; MTG). The PBs were air dried onto the glass slides and FISH was performed, as mentioned previously, with the exception that eight probes were used, namely for chromosomes X, 13, 15, 16, 17, 18, 21, and 22.

The FISH analysis consisted of two consecutive hybridizations following previously published protocols (28). Fluorescence in situ hybridization signals were analyzed applying the filter set recommended by Abbott by two independent observers not later than 20 hours after insemination to allow selection of suitable pronuclear oocytes for further culture before syngamy occurred in accordance with the German Embryo Protection Act. For the purpose of this study, only first PB results from the first PGS cycle were compared with the first PB results from the second PGS cycle.

Outcome Measures and Statistical Considerations

The incidence of euploid oocytes per oocyte with a FISH result was calculated separately for each PGS cycle to account for multiplicity associated with multiple oocytes per woman in a cycle and the resultant percentage values were treated as a continuous variable. Histograms were used for visual evaluation of the distribution of continuous variables. Continuous variables with normal distribution were presented with mean and SD, whereas other continuous variables were defined with median and interquartile range. Depending on the distribution characteristics paired samples *t*-test or related samples Wilcoxon signed rank test was used for comparisons between the results of two consecutive PGS cycles. Categorical variables were defined with percentages.

A linear regression analysis was done to evaluate the effect of female age (adjusted for treatment center) on the difference between euploidy rates in two consecutive cycles. The difference in euploidy rates between two PGS cycles was included as the dependent variable, treatment center, and female age at the time of first treatment cycle (categorized as ≤34 years, 35–39 years, and ≥40 years) as the independent variables. A second regression model involved the absolute difference in the number of euploid oocytes between two cycles as the dependent variable.

The absolute difference between the number of euploid oocytes one woman had in a particular cycle, by age categories, was calculated and presented to allow the reader with practical information useful to inform women in a clinical setting. The minimum clinically significant difference was regarded to be ± one euploid oocyte between two consecutive cycles.

RESULTS

Intercenter Differences: FISH Data

A total of 226 patients from the Italian and German centers were included in the study. The average maternal age of the investigated patient group was 38.2 years (range, 26–47 years). Patient characteristics are summarized in Table 1. As expected female age and the number of previously failed

TABLE 1

Characteristics of the first and second cycles.

	Italy		Germany		Overall	
	1st cycle	2nd cycle	1st cycle	2nd cycle	1st cycle	2nd cycle
No. of women	161	161	65	65	226	226
Age (y) ^a	38.2 ± 0.3	38.9 ± 0.3	37.6 ± 0.3	37.9 ± 0.3	38.0 ± 0.2	38.6 ± 0.2
No. of previously failed cycles ^a	1.7 ± 0.15	2.8 ± 0.15	1.7 ± 0.27	2.3 ± 0.29	1.7 ± 0.13	2.8 ± 0.13
No. of oocytes with FISH result ^d	5.0 (4.0–7.5)	6.0 (4.0–7.0)	5.0 (4.0–7.0)	5.0 (4.0–7.0)	5.0 (4.0–7.0)	6.0 (4.0–7.0)
% Normal per oocyte with FISH result ^d	50.0 (33.3–60.0)	50.0 (33.3–60.0) ^e	16.7 (0–26.8)	16.7 (0–26.1)	42.9 (24.3–57.1)	37.5 (22.2–55.6) ^f
			P value	P value	P value	P value
			<.01 ^b	<.01 ^b	<.01 ^b	<.01 ^b
			<.01 ^b	<.01 ^b	<.01 ^b	<.01 ^b
			.59 ^c	.41 ^c	.41 ^c	.41 ^c
			.34 ^c	.85 ^c	.85 ^c	.37 ^c

^a Values are mean ± standard error of mean.
^b Paired samples *t*-test.
^c Related samples Wilcoxon signed rank test.
^d Median and interquartile range.
^e Includes 160 women as one woman had no oocytes with FISH result.
^f Includes 451 cycles as one woman had no oocytes with FISH result in the second cycle.
 Munné. Intra-age euploidy differences. *Fertil Steril* 2012.

cycles were significantly different between two cycles; however, the mean absolute difference of 0.6 years in age was not expected to affect euploidy rates substantially. Overall, there were no statistically significant differences in any parameter between consecutive cycles conducted at the same center, including euploidy rates, which were similar for cycle 1 and cycle 2. However, despite similar age of the Italian and German women (38.3 ± 3.8 and 37.7 ± 2.7 years, respectively, $P=.2$), significantly ($P<.001$) more euploid oocytes were collected from the Italian (median value of 50% for two cycles combined) group, compared with the German group (median value of 16.7% for two cycles combined). It is important to note that the PBs biopsied from oocytes collected at the German center were analyzed with two extra probes (for chromosomes X and 17), whereas this was not the case for the PBs biopsied from oocytes collected in the Italian center (chromosomes 13, 15, 16, 18, 21, and 22 analyzed only). Of the 681 PBs analyzed in the German center, 22 were abnormal for chromosomes X and/or 17 only. Thus, if we consider these as normal for purposes of comparison with the Italian center, the increased euploidy rate would become 26.3% instead of 16.7%. This rate is still significantly different ($P<.001$) compared with the Italian group (50.0%). In addition, more losses (either chromatid or chromosome) (75%) were observed in the German group of PBs than in the Italian group (54.5%) ($P<.001$).

The analyses carried out by the Italian group included 43 oocytes assessed along with their corresponding PBs. These were used to determine the error rate of the technique. Of these PBs, 8 were classified by PGS as normal and 35 as abnormal (Table 2). After analysis of the associated oocytes using the same method and FISH probes, all normal oocytes were confirmed normal, and 34/35 abnormal oocytes were confirmed abnormal. The one discordant result had a PB1 classified as disomy 22, but the oocyte was shown to be normal. Thus the error rate was 2.3%.

Intra-age Group Differences

In total, the median incidence of euploid oocytes in two consecutive cycles combined was 50% for patients <35 years old, 40% for patients 35–39 years, and 33.3% for patients 40 years and older. However, the intra-age group variation in the proportion of euploid oocytes was large. Within the same age category, although some women had 0 euploid oocytes, other women had 100% euploid oocytes. The incidence of euploid oocytes per cycle across categories of female age and treating center is shown in Table 3.

Overall, 75.6% of women had a difference of ≥ 1 in the number of euploid oocytes between consecutive cycles (77.0% for the Italian group and 72.3% for the German group). This figure ranged from 68.9%–85.7% across age groups (Table 4).

Intercycle Differences: FISH Data

Table 1 shows that there are no significant differences between the first and second cycle regarding number of eggs produced or days of stimulation either in the Italian or

TABLE 2

Error rate from comparing PB and oocyte analysis.

No. of abnormal PB	PB ploidy	Oocyte ploidy	Concordance	Discordance
1	L 22	G 22	1	0
2	L 21, 22	G 21, 22	1	0
3	G 15	L 15	1	0
4	G 22	L 22	1	0
5	L16	G 16	1	0
6	L 22	G 22	1	0
7	G 22	L 22	1	0
8	L 21	G 21	1	0
9	L18	G 18	1	0
10	G 21	L 21	1	0
11	L 22	N	0	1
12	L 21	G 21	1	0
13	L 18	G 18	1	0
14	G 13	L 13	1	0
15	L 16	G 16	1	0
16	G 16	L 16	1	0
17	L 22	G 22	1	0
18	L 21	G 21	1	0
19	L 21	G 21	1	0
20	L 13, 21	G 13, 21	1	0
21	G 13	L 13	1	0
22	L 16	G 16	1	0
23	G 13	L 13	1	0
24	G 13, L 21	L 13, G 21	1	0
25	L 15	G 15	1	0
26	L 22	G 22	1	0
27	L 22	G 22	1	0
28	L 15, 21	G 15, 21	1	0
29	L 15	G 15	1	0
30	L 18	G 18	1	0
31	L 18	G 18	1	0
32	L 22	G 22	1	0
33	L15	G 15	1	0
34	G 21	L 21	1	0
35	L 21	G 21	1	0
Total %			34	1
				2.90%

Note: G = chromosome or chromatid gain; L = chromosome or chromatid loss; N = normal; PB = polar body.

Munné. Intra-age euploidy differences. *Fertil Steril* 2012.

German data sets. On average, only 7 months passed from the first to the second PGS cycle.

Overall, mean oocyte euploidy rates were not statistically significantly different between two subsequent PGS cycles (mean difference 1.04%, 95% confidence interval [CI] -2.48% – 4.56% , $P=.56$). However, the absolute difference between euploidy rates observed in two consecutive cycles of the same women varied widely. The 10th and 90th percentile values of the absolute difference between euploidy rates in two consecutive cycles (euploidy rate in the second cycle subtracted from the euploidy rate in the first cycle) ranged from -33.0% to $+33.0\%$. In some cases euploidy rates were identical in the first cycle and the second (0 variation), whereas in other cases a 100% difference was observed (i.e., 0 euploidy in the first cycle and 100% euploidy in the second cycle [$+100\%$], or vice versa [-100%]). This wide range and unpredictability of euploidy rate was seen in all maternal age groups. As shown in Table 3, the difference between the first and the second cycles did not consistently involve a decrease or an increase in euploidy—the direction of the change was

TABLE 3

Euploidy frequency in the two cycles of the same patient, by age group and center.

	No. of women (%)	% Euploid oocytes in the 1st cycle ^a	% Euploid oocytes in the 2nd cycle ^a	Median difference between euploidy rates in consecutive cycles ^b
Italy				
≤34 y	27 (16.8)	56.2% (50.0, 50.0%–71.4%)	58.1% (60.0, 40.0%–66.7%)	1.9% (5.6, –80%–67%)
35–39 y	70 (43.5)	48.8% (50.0, 39.4%–60.0%)	49.0% (48.3, 33.3%–60.6%)	–0.2% (2.5, –80%–60%)
≥40 y	64 (39.8)	45.3% (43.7, 28.6%–61.9%)	41.0% (42.9, 28.6%–54.2%)	4.3% (0, –75%–75%)
Total	161	48.7% (50.0, 33.3%–60.0%)	47.3 (50.0, 33.3%–60.0%)	1.3% (2.5, –80%–75%)
Germany				
≤34 y	7 (10.8)	24.3% (20.0, 0–50.0%)	23.2% (12.5, 0–57.1%)	–0.02% (–5.0, –67%–100%)
35–39 y	45 (69.2)	17.8% (16.7, 0–28.6%)	19.6% (20.0, 0–31.0%)	–0.7% (–3.0, –50%–67%)
≥40 y	13 (20.0)	13.4% (14.3, 5.6%–20.8%)	4.5% (0, 0–7.1%)	5.2% (3.0, –25%–17%)
Total	65	17.6% (16.7, 0–26.8%)	16.9 (16.7, 0–26.1%)	0.5% (0, –67%–100%)
Overall				
≤34 y	34 (15.0)	49.6% (50.0, 33.3%–62.8%)	50.9% (57.1, 32.5%–66.7%)	–1.5% (0, –80%–100.0%)
35–39 y	115 (50.9)	36.7% (40.0, 20.0%–54.6%)	37.5% (33.3, 22.5%–50.0%)	–0.4% (0, –80%–67.0%)
≥40 y	77 (34.1)	39.9% (37.5, 25.0%–55.8%)	34.8% (33.3, 7.1%–50.0%)	4.5% (3.0, –75%–75%)
Total	226	39.7% (42.9, 24.3%–57.1%)	38.6% (37.5, 22.2%–55.6%)	1.1% (0, –80%–100%)

^a Values are mean (median, interquartile range).
^b Values are mean (median, minimum – maximum).

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random. This phenomenon was observed for both the Italian and German data sets across all three age groups.

Regression analysis showed that female age, adjusted for treatment center, did not affect the difference between euploidy rates in two consecutive cycles (Beta: 3.37%, 95% CI: –2.0%–8.8%, *P* = .22 for age group). Overall, the majority of patients (68.5%) had less than ±2 euploid eggs of difference between cycles. As a result, 76.9% and 65.2% patients from the German and Italian centers, respectively, had less than ±2 euploid eggs of difference between cycles (Table 4).

DISCUSSION

The present study involved FISH for the analysis of first PBs. Fluorescence in situ hybridization requires that PBs are fixed onto microscope slides, a procedure that could lead to the artifactual loss of chromosome material. An excess of missing chromosomes or chromatids in both first and second PBs has been observed in some studies (25, 29). Comparative genomic hybridization (CGH) and array CGH is not affected by cell fixation or signal overlap, but most studies on PBs have also detected an excess of monosomies (30, 31). This

TABLE 4

Absolute difference between the number of euploid oocytes in two consecutive cycles.

	Absolute difference in no. euploid oocytes	Italy No. of women (%)	Germany No. of women (%)	Total No. of women (%)
Overall				
Mean no. of euploid oocytes in subgroup		2.8	1.0	2.3
	0	37 (23.0)	18 (27.7)	55 (24.3)
	1	68 (42.2)	32 (49.2)	100 (44.2)
	≥2	56 (34.8)	15 (23.1)	71 (31.4)
≤34 y				
Mean no. of euploid oocytes in subgroup		3.0	1.4	2.7
	0	8 (29.6)	1 (14.3)	9 (26.5)
	1	10 (37.3)	4 (57.1)	14 (41.2)
	≥2	9 (33.3)	2 (28.6)	11 (14.4)
35–39 y				
Mean no. of euploid oocytes in subgroup		3.0	1.1	2.2
	0	10 (14.3)	14 (31.1)	24 (20.9)
	1	34 (48.6)	21 (46.7)	55 (47.8)
	≥2	26 (37.1)	10 (22.2)	36 (31.3)
≥40 y				
Mean no. of euploid oocytes in subgroup		2.4	0.7	2.1
	0	19 (29.7)	3 (23.1)	22 (28.6)
	1	24 (37.5)	7 (53.8)	31 (40.3)
	≥2	21 (32.8)	3 (23.1)	24 (31.2)

Note: Regression analysis revealed that the absolute difference in the number of euploid oocytes between two preimplantation genetic screening cycles was not dependent on maternal age (*P* = .13) or on treating center (*P* = .11).

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agreement between array CGH and FISH results suggests that the losses of chromosome material scored during FISH PB analysis are not likely to be an artifact of the spreading process, but may represent a genuine biological phenomenon (30).

Another disadvantage of FISH is that only a few chromosomes can be analyzed simultaneously. Conversely, CGH can provide a complete molecular karyotype and has been successfully used to analyze PBs (30, 32–35). An improvement to CGH is microarray CGH, which produces lower error rates (1.8%) (36) than FISH (4.7%) (37) in embryos, and oocytes (6% for all chromosomes by array CGH vs. 6% for 5 chromosomes by FISH) (38, 39). Comprehensive chromosome analysis should result in a better assessment of the number of viable oocytes obtained from a given ovarian stimulation cycle. Chromosomally normal blastocysts, as determined using CGH, quantitative Polymerase Chain Reaction (PCR), or SNP arrays, have been reported to implant with a 60% rate or higher and seldom miscarry in women of advanced maternal age (6, 7, 39–43). It should be possible to determine the number of oocytes required to provide a reasonable chance of live birth using estimates of blastocyst formation rate per euploid oocyte and blastocyst implantation rate in a particular assisted reproductive technology (ART) laboratory.

Pituitary suppression was achieved with the long GnRH agonist protocol in all PGS cycles, but gonadotropin dosage was not fixed. Although this can be considered a limitation for the current study, this is indeed reflective of real life because not only the gonadotropin dosage but even the pituitary suppression protocol can be changed for a subsequent cycle depending on the outcome of the initial one as well as on other conditions (e.g., scheduling requirements). In addition, the number of oocytes collected varied little between cycles (Table 1). A previous study showed that collection of more oocytes in a second cycle was potentially associated with lower aneuploidy rates than that in the first cycle (20). However, in the present study the number of oocytes collected and aneuploidy rates were both similar between the first and the second cycles.

The study patient group was generally of advanced reproductive age (35 years or older). The relatively high aneuploidy rate seen was therefore not unexpected. However, some egg donation cycles studied in the past, at the embryo level, have also shown high rates of chromosome abnormalities in some donors (19), and thus variability between patients within the same age group seems not to be limited to patients of advanced reproductive age. However, a recent study applying CGH to PBs from donor oocytes identified very little variability, although this could be explained by the low aneuploidy rate found in that investigation (34).

Finally, this approach analyzes only first polar bodies and does not account either for chromosome abnormalities arising during the second meiotic division, or for aneuploidy of post-zygotic origin. It can be argued that the first PB analysis alone is not enough to have a good chromosomal assessment of the oocyte and that at least first and second PBs should be analyzed (44, 45). This is because the majority of first meiosis nondisjunction events have been confirmed to be chromatid and not whole chromosome errors with three different

techniques (karyotype, FISH, and recently array CGH) (46–48). This means that oocytes determined to be abnormal in their first meiotic division can sometimes be “corrected” by a second meiotic division error (48). However, embryo analysis of “corrected” embryos showed that the majority were composed of cells with multiple mitotic chromosome errors (chaotic mosaics) (49) and thus correction to euploidy seems unlikely.

Intra-age Group and Intercenter Differences

Despite the well-documented increase in chromosome abnormalities in oocytes and embryos with advancing maternal age (2, 3, 25, 30, 42, 44, 48, 50, 51), also confirmed in the present study, this study reports dramatic intra-age group differences in aneuploidy rates. This suggests that age is only a gross indicator for euploidy, providing only a very rough estimate of the frequency of abnormalities. Apparently patients of similar age may have very different proportions of euploid oocytes. In addition to interage differences, we observed substantial variation between centers. This variation could be due to the use of different stimulation protocols (21, 52, 53) and/or other technical differences in the IVF procedure (54), or in the FISH technique, as discussed previously. In absolute number of euploid eggs in comparison to the mean number of euploid eggs per each age group and center, the variability does not appear to be as big, with 31.4% of patients having ± 2 or more euploid eggs than the average.

Intercycle Differences

The present data indicate large differences in euploidy rates between cycles of the same patient, ranging from -100% to $+75\%$ points difference between cycles. Intercycle variation existed in both centers and was not dependent on maternal age. Thus, PB euploidy rates in the first cycle are not predictive of euploidy rates in the second cycle. It is still possible that more comprehensive chromosome analysis, such as array CGH, could produce better results than FISH and improve prediction for any subsequent cycles from the results obtained after the first cycle.

Repercussions for FP

Based on these results the premise that FP can be achieved based on freezing a set number of oocytes depending on a woman's age is not valid. Other factors have been reported to influence the incidence of aneuploidy besides age, namely the type of response to hormonal stimulation, the cause of infertility, and poor prognosis (25), but these are even less predictive of outcome than maternal age.

Another important factor that makes predicting euploidy based on maternal age across patients even more challenging is the difference in euploidy rates observed between centers. The differences in euploidy frequency between patients attending the two centers contributing to this study were highly significant, and thus rules for the whole infertility field about how many oocytes should be frozen depending on maternal age are not appropriate. The results of this study indicate that such guidelines (currently nonexistent) would need to

be tailored to each IVF center depending to its underlying aneuploidy rate for a certain age.

An alternative to freezing ever increasing numbers of oocytes with increasing maternal age to compensate for those that are expected to be chromosomally abnormal is to test the oocytes by first PB analysis to determine how many euploid oocytes would be available (27, 54). The study of Keskindepe et al. (54) showed that first PB removal before egg preservation is safe and does not interfere with egg survival. An ongoing clinical randomized study organized by the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium will determine whether PB biopsy and array CGH can improve pregnancy results. If so, PB analysis of eggs in each FP could be a better estimator of how many eggs need to be frozen, than maternal age alone, or even analyzing only the first FP cycle with PGS.

REFERENCES

- Society Assisted Reproductive Technology (SART). National summary. Available at: http://www.sart.org/find_frm.html. Accessed June 1, 2011.
- Munné S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995;64:382–91.
- Dailey T, Dale B, Cohen J, Munné S. Association between non-disjunction and maternal age in meiosis-II human oocytes detected by FISH analysis. *Am J Human Genet* 1996;59:176–84.
- Sauer MV, Paulson RJ, Lobo RA. Reversing the natural decline in human fertility. An extended clinical trial of oocyte donation to women of advanced reproductive age. *JAMA* 1992;268:1275–9.
- Wells D, Fragouli E, Alfarawati S, Munne S, Schoolcraft W, Katz-Jaffe M. Highly significant improvement in embryo implantation and increased live birth rate achieved after comprehensive chromosomal screening: implications for single embryo transfer. *Fertil Steril* 2009;92:S79.
- Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. *Fertil Steril* 2011;96:638–40.
- Scott RT, Tao X, Taylor D, Ferry KM, Treff NR. A prospective randomized controlled trial demonstrating significantly increased clinical pregnancy rates following 24 chromosome aneuploidy screening: biopsy and analysis on day 5 with fresh transfer. *Fertil Steril* 2010;94:S2 (O-05).
- Schoolcraft WB, Fragouli E, Stevens J, Munné S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril* 2010;94:1700–6.
- Kuleshova L, Gianaroli L, Magli MC, Ferraretti AP, Trounson AO. Birth following vitrification of a small number of human oocytes. *Hum Reprod* 1999;14:3077–9.
- Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online* 2005;11:300–8.
- Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril* 2009;92:520–6.
- Grifo J, Noyes N. Delivery rates using cryopreserved oocytes is comparable to conventional in vitro fertilization using fresh oocytes: potential fertility preservation for female cancer patients. *Fertil Steril* 2009;93:391–6.
- Cobo A, Meseguer M, Remoh J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Hum Reprod* 2010;25:2239–46.
- Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh “versus” vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. *Hum Reprod* 2010;25:66–73.
- Cobo A, Domingo J, Perez S, Crespo J, Remohi J, Pellicer A. Vitrification, an effective new approach to oocyte banking in healthy women, could be applied in cancer patients to preserve their fertility. *Clin Transl Oncol* 2008;10:268–73.
- Noyes N, Labella PA, Grifo J, Knopman JM. Oocyte cryopreservation: a feasible fertility preservation option for reproductive age cancer survivors. *J Assist Reprod Genet* 2011;27:495–9.
- Homburg R, van der Veen F, Silber SJ. Oocyte vitrification—women’s emancipation set in stone. *Fertil Steril* 2009;91(Suppl 4):1319–20.
- Rudick R, Opper N, Paulson R, Bendikson K, Chung K. The status of oocyte cryopreservation in the United States. *Fertil Steril* 2010;94:2642–6.
- Munné S, Ary J, Zouves C, Escudero T, Barnes F, Cinioglu C, et al. Wide range of chromosome abnormalities in the embryos of young egg donors. *Reprod Biomed Online* 2006;12:340–6.
- Munné S, Escudero T, Pere C, Xuezhong Z, Oter M, Garrisi M, et al. Predictability of preimplantation genetic diagnosis of aneuploidy and translocations on prospective attempts. *Reprod Biomed Online* 2004;9:645–51.
- Munné S, Magli C, Adler A, Wright G, de Boer K, Mortimer D, et al. Treatment-related chromosome abnormalities in human embryos. *Hum Reprod* 1997;12:780–4.
- Ferraretti AP, Gianaroli L, Magli MC, D’Angelo A, Farfalli V, Montanaro N. Exogenous LH in COH for ART: when and which? *Fertil Steril* 2004;82:1521–6.
- Ferraretti AP, Magli MC, Feliciani E, Montanaro N, Gianaroli L. Relationship of timing agonist administration in the cycle phase to the ovarian response to gonadotropins in the long-down regulation protocols for assisted reproductive technologies. *Fertil Steril* 1996;65:114–21.
- Magli MC, Ferraretti AP, Crippa A, Lappi M, Feliciani E, Gianaroli L. First meiosis errors in immature oocytes generated by stimulated cycles. *Fertil Steril* 2006;86:629–35.
- Gianaroli L, Magli MC, Cavallini G, Crippa A, Capoti A, Resta S, et al. Predicting aneuploidy in human oocytes: key factors which affect the meiotic process. *Hum Reprod* 2010;25:2374–86.
- Montag M, van der Ven K, Dorn C. Outcome of laser-assisted polar body biopsy and aneuploidy testing. *Reprod BioMed Online* 2004;9:425–9.
- Naether OGJ, Rudolf K, Fischer R, Baukloh V, Schmidt C, Held K. Pregnancy after vitrification of pronuclear stage oocytes biopsied for polar body aneuploidy. *Reprod Biomed Online* 2008;16:268–70.
- Munné S, Magli C, Bahçe M, Fung J, Legator M, Morrison L, et al. Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. *Prenatal Diagn* 1998;18:1459–66.
- Kuliev A, Cieslak J, Verlinsky Y. Frequency and distribution of chromosome abnormalities in human oocytes. *Cytogenet Genome Res* 2005;111:193–8.
- Fragouli E, Alfarawati S, Goodall NN, Sánchez-García JF, Colls P, Wells D. The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. *Mol Hum Reprod* 2011 Apr 14. [Epub ahead of print.]
- Magli MC, Montag M, Köster M, Muzi L, Geraedts J, Collins J, et al. Polar body array CGH for prediction of the status of the corresponding oocyte. Part II: technical aspects. *Hum Reprod* 2011. doi: 10.1093/humrep/der294.
- Gutiérrez-Mateo C, Wells D, Benet J, Sánchez-García JF, Bermúdez MG, Belil I, et al. Reliability of comparative genomic hybridization to detect chromosome abnormalities in first polar bodies and metaphase II oocytes. *Hum Reprod* 2004;19:2118–25.
- Sher G, Keskindepe L, Keskindepe M, Ginsburg M, Maassarani G, Yakut T, et al. Oocyte karyotyping by comparative genomic hybridization provides a highly reliable method for selecting “competent” embryos, markedly improving in vitro fertilization outcome: a multiphase study. *Fertil Steril* 2007;87:1033–40.
- Fragouli E, Escalona A, Gutierrez-Mateo C, Tormasi S, Alfarawati S, Sepulveda S, et al. Comparative genomic hybridization of oocytes and first polar bodies from young donors. *Reprod Biomed Online* 2009;18:228–37.
- Fragouli E, Alfarawati S, Katz-Jaffe M, Stevens J, Colls P, Goodall N, et al. Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. *Fertil Steril* 2010;94:875–87.
- Gutiérrez-Mateo C, Colls P, Sánchez-García J, Escudero T, Prates R, Ketterson K, et al. Validation of microarray comparative genomic

- hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril* 2011;95:953–8.
37. Colls P, Escudero T, Cekleniak N, Sadowy S, Cohen J, Munné S. Increased efficiency of preimplantation genetic diagnosis for infertility using “no result rescue.” *Fertil Steril* 2007;88:53–61.
 38. Magli MC, Grugnetti C, Castelletti E, Paviglianiti B, Ferraretti AP, Geraedts J, et al. Five chromosome segregation in polar bodies and the corresponding oocytes. *Reprod Biomed Online* 2012;24:331–8.
 39. Geraedts J, Montag M, Magli MC, Repping S, Handyside A, Staessen C, et al. Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results. *Hum Reprod* 2011;26:3173–80.
 40. Munné S, Hill D, Wagner C, Ketterson K, Cohen J, Wells D. Pregnancy outcome following comprehensive chromosome analysis at the cleavage and blastocyst stages. *Fertil Steril* 2010;94:52.
 41. Forman EJ, Treff NR, Tao X, Ferry KM, Taylor D, Scott RT. Comprehensive chromosome screening (CCS) results in significantly higher pregnancy rates and lower loss rates from single embryo transfer (SET) in a poor prognosis population. *Fertil Steril* 2011;96(Suppl 1):O-58.
 42. Grifo J, Ghadir S, Kaplan B, Laskin CA, Glassner M, Munné S. Significant decrease in miscarriages after preimplantation genetic diagnosis (PGD) for recurrent pregnancy loss using array comparative genome hybridization (Array CGH). *Fertil Steril* 2011;96(Suppl 1):O-76.
 43. Harton G, Surrey M, Grifo J, Kaplan B, Ahlering P, Cohen J. Implantation of euploid blastocysts, assessed by array comparative genomic hybridization (aCGH) in unstimulated cycles is not correlated with maternal age. *Fertil Steril* 2011;96(Suppl):O-183.
 44. Ferraretti AP, Magli MC, Kopcow L, Gianaroli L. Prognostic role of preimplantation genetic diagnosis for aneuploidy in assisted reproduction technology outcome. *Hum Reprod* 2004;19:694–9.
 45. Gabriel AS, Thornhill AR, Ottolini CS, Gordon A, Brown APC, Taylor J, et al. Array comparative genomic hybridization on first polar bodies suggests that non-disjunction is not the predominant mechanism leading to aneuploidy in humans. *J Med Genet* 2010. doi: 10.1136/jmg.2010.088070.
 46. Angell RR. Predivision in human oocytes at meiosis I: a mechanism for trisomy formation in man. *Hum Genet* 1991;86:383–7.
 47. Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y. Chromosomal abnormalities in a series of 6733 human oocytes in preimplantation diagnosis for age-related aneuploidies. *Reprod Biomed Online* 2002;6:54–9.
 48. Kuliev A, Verlinsky Y. Meiotic and mitotic non-disjunction: lessons from preimplantation genetic diagnosis. *Hum Reprod Update* 2004;10:401–7.
 49. Munné S, Chen S, Colls P, Garrisi J, Zheng X, Cekleniak N, et al. Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. *Reprod Biomed Online* 2007;14:628–34.
 50. Magli MC, Gianaroli L, Ferraretti AP. Chromosomal abnormalities in embryos. *Mol Cell Endocrinol* 2001;22(183 Suppl 1):S29–34.
 51. Jackson KV, Ginsburg ES, Hornstein D, Rein MS, Clarke RN. Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization-embryo transfer cycles. *Fertil Steril* 1998;70:60–6.
 52. Weghofer A, Munné S, Brannath W, Chen S, Tomkin G, Cekleniak N, et al. The impact of LH-containing gonadotropins on diploidy rates in preimplantation embryos: long protocol stimulation. *Hum Reprod* 2008;23:499–503.
 53. Weghofer A, Munné S, Brannath W, Chen S, Barad D, Cohen J, et al. The impact of LH-containing gonadotropin stimulation on euploidy rates in preimplantation embryos: antagonist cycles. *Fertil Steril* 2009;92:937–42.
 54. Keskinetepe L, Agca Y, Sher G, Keskinetepe M, Maassarani G. High survival rate of metaphase II human oocytes after first polar body biopsy and vitrification: determining the effect of previtrification conditions. *Fertil Steril* 2009;92:1706–15.