Pregnancy outcomes from more than 1,800 in vitro fertilization cycles with the use of 24-chromosome single-nucleotide polymorphism–based preimplantation genetic testing for aneuploidy

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Objective: To measure in vitro fertilization (IVF) outcomes following 24-chromosome single–nucleotide-polymorphism (SNP)–based preimplantation genetic testing for aneuploidy (PGT-A) and euploid embryo transfer.
Design: Retrospective.
Setting: Fertility clinics and laboratory.
Patient(s): Women 20–46 years of age undergoing IVF treatment.
Intervention(s): Twenty-four-chromosome SNP-based PGT-A of day 5/6 embryo biopsies.
Main Outcome Measure(s): Maternal age–stratified implantation, clinical pregnancy, and live birth rates per embryo transfer; miscarriage rates; and number of embryo transfers per patient needed to achieve a live birth.
Result(s): An implantation rate of 69.9%, clinical pregnancy rate per transfer of 70.6%, and live birth rate per transfer of 64.5% were observed in 1,621 nondonor frozen cycles with the use of SNP-based PGT-A. In addition, SNP-based PGT-A outcomes, when measured per cycle with transfer, remained relatively constant across all maternal ages; when measured per cycle initiated, they decreased as maternal age increased. Miscarriage rates were ~5% in women ≤40 years old. No statistically significant differences in pregnancy outcomes were found for single-embryo transfers (SET) versus double-embryo transfers with SNP-based PGT-A. On average, 1.38 embryo transfers per patient were needed to achieve a live birth in nondonor cycles.
Conclusion(s): Our findings that SNP-based PGT-A can mitigate the negative effects of maternal age on IVF outcomes in cycles with transfer, and that pregnancy outcomes from SET cycles are not significantly different from those of double-embryo transfer cycles, support the use of SET when transfers are combined with SNP-based PGT-A. (Fertil Steril® 2018;110:113–21. ©2018 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)
This abstract is available in Spanish at the end of the article.

Key Words: Aneuploidy, in vitro fertilization, preimplantation genetic testing for aneuploidy, pregnancy outcome, single-nucleotide polymorphism

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Despite recent advances in embryology research and assisted reproduction technology, clinical outcome measures for in vitro fertilization (IVF) treatment (e.g., implantation, pregnancy, and live birth rates) remain relatively low. Currently, in the U.S., fewer than half of all transferred embryos implant and lead to successful pregnancy, regardless of maternal age (1, 2). Although the likelihood of a live birth can be increased by transferring more than one embryo per IVF cycle, this increases the likelihood of multiple pregnancies and their attendant medical risks, including pregnancy complications and perinatal morbidity and mortality (3–5), and increases financial and psychosocial burdens (6, 7). Instead, methods that improve per-embryo implantation rates would be preferable.

Aneuploidy, defined as an abnormal number of chromosomes in a genome, is common in human embryos (8–11)—particularly in women of advanced maternal age (>35 y) (12)—and is the primary cause of failed IVF cycles (13–15). Preimplantation genetic testing for aneuploidy (PGT-A) of embryos coupled with selective transfer of euploid embryos has the potential to improve implantation rates and pregnancy outcomes in patients undergoing IVF treatment (16). However, accurate determination of embryo ploidy is critical. Fertility specialists generally agree that comprehensive chromosome screening (CCS) is superior to screening with the use of fluorescence in situ hybridization, and that performing PGT-A on a small number of trophectoderm (TE) cells from day 5 blastocyst-stage embryos is preferable to analysis of a single blastomere cell from day 3 cleavage-stage embryos (17, 18). CCS can be performed by several methods, including quantitative polymerase chain reaction (qPCR), array comparative genomic hybridization (aCGH), single-nucleotide polymorphism (SNP)–based microarray, and next-generation sequencing (NGS) (17, 19).

SNP-based PGT-A has been shown to be as accurate as metaphase karyotyping, historically the criterion standard technique for chromosome analysis (20). However, large studies focusing on IVF outcomes when using SNP-based PGT-A are lacking. Advantages of the SNP-based microarray method include the ability to detect triploidy and haploidy (10, 11), embryo fingerprinting, and determination of parental origin of aneuploidy. Here, we present age- and egg donor–stratified outcome measures, including implantation, clinical pregnancy, and live birth rates, from more than 1,800 IVF cycles performed with the use of SNP-based PGT-A.

MATERIALS AND METHODS

This was a retrospective study of pregnancy outcomes of IVF procedures performed at Pacific Fertility Center (PFC; San Francisco) and Conceptions Reproductive Associates of Colorado (CRA; Littleton) from October 1, 2010, to August 31, 2013. Women 18–55 years of age who underwent IVF treatment at these centers were eligible for inclusion. Indications for PGT-A included diminished ovarian reserve, male-factor infertility, uterine-factor infertility, tubal disease, polycystic ovarian syndrome, endometriosis, and idiopathic infertility. Patients who did not elect 24-chromosome SNP-based PGT-A were excluded.

All patients provided informed consents for analyses of their deidentified data before undergoing IVF procedures. An independent Institutional Review Board, Schulman IRB (Cincinnati), granted PFC an exemption for this study (no. CCS2180) because the work was not considered to involve human subjects.

In Vitro Fertilization

Controlled ovarian stimulation, oocyte retrieval, insemination, embryo culture, embryo grading, TE biopsy, embryo vitrification/warming, and embryo transfer were performed according to standard operating procedures, as described below. Both fertility centers followed similar procedures, except where noted.

Ovarian Stimulation and Oocyte Retrieval

Ovarian stimulation was performed using one of five different protocols, depending on physician preference and patient characteristics. At CRA, both egg donors and patients undergoing IVF with their own eggs (hereafter referred to as "nondonors") were treated with the use of a GnRH antagonist protocol with a dual trigger of hCG (Noravel, Ferring; or Pregnyl, Merck) and leuprolide acetate (21) (Lupron, Abbvie), after pretreatment with the use of oral contraceptives or oral luteal-phase 17β-E_2 (Estrace, Allergan).

At PFC, egg donors and nondonors were treated with a long-luteal GnRH-agonist protocol (22) or an antagonist protocol (23), with or without pretreatment with oral contraceptives. Some nondonors with decreased ovarian reserve were also treated with the use of a microdose agonist "flare" protocol (23, 24) with or without pretreatment with oral luteal-phase 17β-E_2. Subsequently, oocytes were retrieved from all patients with at least one mature follicle 36 hours after administration of hCG.

Oocyte Insemination

Oocyte insemination was performed by either conventional IVF microdrop insemination 2–6 hours after oocyte collection or by intracytoplasmic sperm injection (ICSI).

Embryo Culture

At CRA, embryos were cultured in Sequential Series culture medium with 10% Quinn Advantage Serum Protein Substitute (Origio, Trumbull) until the blastocyst stage in a humidified gas-controlled incubator (Cook Medical; Panasonic Healthcare). At PFC, embryos were cultured in Continuous Single Culture growth medium with 10% Serum Substitute Supplement (Irvine Scientific) until the blastocyst stage in a humidified gas-controlled incubator (Thermo Scientific). Incubators were kept at 37°C with 5% oxygen, 6%–7% carbon

dioxide (adjusted to achieve a pH of 7.2–7.4), and nitrogen balance.

Embryo Grading and Trophectoderm Biopsy

Embryos were graded according to their degree of fragmentation, symmetry, and quality of the inner cell mass (ICM) and TE as described previously (25, 26). High- and medium-grade embryos had a large structured ICM and a distinct continuous layer of trophoblasts that were highly symmetric in size and shape, with little or no cellular fragmentation (cytoplasmic blebs). In contrast, low-grade embryos had a small or unstructured ICM and/or indistinct or fragmented trophoblasts.

For SNP-based PGT-A, three to eight TE cells were biopsied from high- and medium-grade embryos on culture day 5 or 6. Biopsy samples were processed and shipped overnight to Natera (San Carlos, California) according to Natera standard operating procedures.

Embryo Vitrification and Warming

Biopsied embryos intended for frozen transfers were vitrified using a closed vitrification system (Vit Kit–Freeze, Irvine Scientific) or Rapid-i Vitrification System (Vitrolife) and stored in liquid nitrogen. Embryos were warmed with the use of Vit Kit–Thaw (Irvine Scientific) or Rapidwarm Blast (Vitrolife), and then cultured for 20–60 minutes before transfer.

Endometrial Preparation and Support

At CRA, all patients underwent office hysteroscopy before embryo transfer. Patients with irregular endometrium or uterine polyps underwent hysteroscopic loop removal. At the time of polypectomy, the remainder of the stratum functionalis was gently removed with the use of a cold loop to allow for synchronous regeneration (27, 28).

After pituitary down-regulation with the use of leuprolide (e.g., Lupron), endometrial proliferation was induced with the use of E_2 transdermal patch, vaginal E_2 (Estrace), or intramuscular E_2 valerate per physician preference. Dose adjustments were made on day 6 based on endometrial thickness and E_2 level. If endometrial thickness was $\geq$8 mm, intramuscular hCG (5,000 U) was administered on day 10 and then intramuscular P commenced on the following day until the 10th week of pregnancy, with the option to switch to vaginal P after a positive pregnancy test.

At PFC, uterine preparation for frozen-thawed embryo transfer was done in either natural (i.e., regular ovulation) or programmed (irregular) cycles. For natural-cycle FETs, patients monitored their urine for LH surges, and received recombinant hCG (rhCG; Ovidrel 250 mcg, EMD Serono) either when a surge was detected as an adjunct to an endogenous LH surge or when ovulation was triggered by rhCG injection and the endometrium and dominant follicle on the midcycle ultrasound appeared to be adequate (e.g., $\geq$8 mm endometrial thickness). Intravaginal micronized P (200 mg) was administered twice daily beginning 2 days after rhCG administration and continuing until the 7th week of pregnancy. Frozen-thawed embryo transfer was performed 1 week after hCG administration.

For programmed cycles, patients received biweekly intramuscular injections of E_2 valerate (4 mg). After three doses, if endometrial thickness was adequate and ovarian quiescence was confirmed, patients added daily intramuscular injections of P in ethyl oleate (50 mg). Frozen-thawed embryo transfer was performed on the 6th day of progesterone administration. Estradiol valerate and P in ethyl oleate were continued until the 10th week of pregnancy.

Preimplantation Genetic Screening

Biopsied TE cells were analyzed by means of 24-chromosome SNP-based PGT-A at Natera, a Clinical Laboratory Improvement Amendments–approved and College of American Pathologists–accredited laboratory. This test involved amplification of genomic DNA from biopsied embryo cells, genotyping with the use of SNP microarrays (Illumina), and determination of copy numbers for all chromosomes, as previously described (20) with the following adaptations to the molecular biology portion of the analysis: Cell biopsies in 5 μL phosphate-buffered saline solution with 0.05% bovine serum albumin (BSA) were combined with 6.1 μL lysis buffer (1.3$\times$ Arcturus Picopure Lysis Buffer, 47 mmol/L KCl, 1 mmol/L $MgCl_2$, 1 mmol/L Tris-HCl pH 7.5, 8 mmol/L dithiothreitol, and a mixture of proprietary primers at 2.5 mmol/L). The samples were incubated at 56°C for 1 hour, 95°C for 10 minutes, and 25°C for 15 minutes. Multiple displacement amplification (MDA) reactions were performed with the use of the Genomiphi V2 DNA Amplification Kit supplemented with 7.5 μg BSA and were incubated at 30°C for 2.5 hours and then 65°C for 10 minutes. On the basis of these results, embryos were classified as either euploid, aneuploid, or no result.

Data Collection and Analyses

Patient databases at each fertility center were queried to obtain the data to compute IVF outcome measures (implantation, clinical pregnancy, live birth, and miscarriage rates). Each of these measures was stratified into the five maternal-age categories defined by the Society for Assisted Reproductive Technologies (SART; <35, 35–37, 38–40, 41–42, and >42 years), and by egg donor status (donor or nondonor). Data were summarized in aggregate and analyzed with the use of Microsoft Excel 2016 and Perl (version 5.18.2) scripts.

Illustrative outcome measures for IVF cycles without PGT-A performed in the U.S. were obtained from the 2014 SART Clinical Outcomes Reporting System National Summary Report (2), using noncumulative data in the "patient's own eggs preliminary primary outcome per intended retrieval" section, which was filtered by frozen cycles (only include frozen embryos and exclude embryos that underwent PGT-A) and by fresh cycles (exclude frozen embryos and exclude embryos that underwent PGT-A).

Pregnancy Outcome Measures

The implantation rate was calculated by dividing the number of gestational sacs observed by means of ultrasound by the number of embryos transferred. The clinical pregnancy rate

per embryo transfer or per retrieval was determined by dividing the number of embryo transfers having a gestational sac observed by means of ultrasound by the number of embryo transfers or egg retrievals, respectively. The live birth rate per embryo transfer or per retrieval was defined as the number of deliveries divided by the number of embryo transfers or egg retrievals, respectively. When not specified, the terms "pregnancy rate" and "live birth rate" refer to rates per embryo transfer. The miscarriage rate was calculated by dividing the number of pregnancies with a fetal heart tone that did not deliver a baby by the number of pregnancies with a fetal heart tone.

RESULTS

Characteristics of Patients and IVF Cycles

During the study period, 974 women (20–46 years of age) underwent 1,883 IVF cycles (total egg retrievals and thaws), of which 1,621 were nondonor cycles and 262 were donor cycles (Table 1). The total number of IVF cycles with PGT-A represented 53.0% (1,883/3,554) of all cycles performed at the participating clinics during the time period of this study; the two clinics had different rates of PGT-A uptake, with CRA performing PGT-A on 81.3% (881/1,084) of cycles and PFC on 40.5% (1,002/2,470) of cycles.

For all cycles, the most common indications for IVF were diminished ovarian reserve (35.8%) and male-factor infertility (22.7%). As expected, the frequency of diminished ovarian reserve increased with maternal age for both nondonor and donor cycles (Table 1; some data not presented).

Aneuploidy Rates

A total of 3,934 blastocysts were biopsied for SNP-based PGT-A. Aneuploidy was detected in 42.9% blastocysts from nondonor cycles (Table 2). As expected, the proportion of aneuploid blastocysts in nondonor cycles increased with maternal age, from 26.9% in women <35 years of age to >70% in women >40 years of age. Likewise, the proportion of egg retrievals and embryo biopsy cases without any euploid embryos increased with maternal age (Table 2).

Embryo Thaws and Transfers

Thaw survival rates were >95% in all age categories (Table 3), and did not differ significantly between CRA and PFC ($P>.05$; Supplemental Tables 1 and 2, available online at www.fertstert.org). These results show that there is minimal loss of embryos before transfer in cycles in which all euploid embryos are vitrified (i.e., freeze-all cycles).

A total of 951 euploid embryos were transferred in 810 transfers, of which 670 (82.7%) were single-embryo transfers (SETs), 139 (17.2%) were double-embryo transfers, and one was a three-embryo transfer (Table 3). The rates of SET at the two centers were different (PFC 91.3% vs. CRA 72.5%; $P=.03$), and the overall SET rate tended to increase with maternal age (range 79.1%–93.1%).

Pregnancy Outcome Measures

Pregnancy outcomes of IVF cycles with the use of SNP-based PGT-A and euploid embryo transfer in this study were relatively high, with low miscarriage rates (Table 3; Supplemental Tables 1 and 2). Specifically, for all nondonor cycles, relatively high implantation (69.9%), clinical pregnancy (70.6%), and live birth (64.5%) rates per transfer were observed, with miscarriages in 4.7% of all pregnancies that had a fetal heart tone (Table 3). Of note, outcome rates per transfer for cycles with the use of SNP-based PGT-A remained relatively constant across all maternal ages (Table 3; Fig. 1). Furthermore, implantation, clinical pregnancy, and live birth rates per transfer from SET and double-embryo transfers were not significantly different (Table 3), although not all age categories could be compared statistically owing to the scarcity of double-embryo transfers in nondonor cycles in women

TABLE 1

Characteristics of patients and in vitro fertilization (IVF) cycles in this study, stratified by egg donor status and maternal age.

		Nondonor (own) eggs					Donor eggs
Characteristic	**All ages[a]**	**< 35 y**	**35–37y**	**38–40y**	**41–42y**	**> 42y**	**All ages[b]**
Patients	866	256	220	227	118	65	108
IVF cycles[c]	1,621	521	404	419	193	84	262
Primary reason for IVF (%)[d]							
Diminished ovarian reserve	33.4	23.8	30.0	36.1	48.3	53.8	55.6
Male-factor infertility	24.0	28.5	30.0	22.5	15.3	7.7	13.9
Polycystic ovarian syndrome	7.0	10.9	7.7	4.8	1.7	4.6	0
Uterine factor infertility	4.6	2.3	3.6	5.7	6.8	9.2	4.6
Tubal disease	3.8	5.5	2.7	2.2	5.1	3.1	0.9
Endometriosis	1.3	1.2	0.9	1.8	1.7	0	3.7
Other infertility condition	12.4	12.9	10.9	14.1	11.9	10.8	7.4
Idiopathic	6.8	9.4	6.4	6.6	4.2	3.1	0
Other (not infertile)	6.1	5.5	7.7	6.2	5.0	1.5	13.0

[a] Nondonor age range 20–46 y.
[b] Donor age range 20–39 y.
[c] Total egg retrievals and thaws.
[d] Percentages do not add up to 100% for all age categories owing to missing data for some cycles.

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TABLE 2

Embryo biopsies and aneuploidy rates in this study, stratified by egg donor status and maternal age.

		Nondonor (own) eggs					Donor eggs
Parameter	**All ages[a]**	**< 35 y**	**35–37y**	**38–40y**	**41–42y**	**> 42y**	**All ages[b]**
Biopsy cases	721	210	188	192	89	42	102
Retrievals	952	264	229	249	144	66	117
Blastocysts biopsied							
Total (n)	3,117	1,093	803	795	293	133	817
No result (n)	42	17	10	10	3	2	16
Aneuploid (n)	1,319	289	300	428	210	92	212
Aneuploid (%)	42.9	26.9	37.8	54.5	72.4	70.2	26.5
Cohorts without euploid embryos (%)[c]							
Biopsy cases	18.4	2.9	11.7	20.3	44.9	61.9	1.0
Retrievals	14.1	2.3	9.6	16.1	27.8	39.4	0.9

[a] Nondonor age range 20–46 y.
[b] Donor age range 20–39 y.
[c] Cohorts without biopsy results were excluded.

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>40 years of age. On average, 1.38 embryo transfers per patient were needed to achieve a live birth in nondonor cycles (Table 3). As expected, because not all retrievals involve an embryo transfer, outcome measures per retrieval decreased with increasing maternal age, regardless of whether PGT-A was performed (Table 3; Fig. 1D and 1E).

DISCUSSION

To our knowledge, this is the largest study of pregnancy outcomes in women undergoing IVF with the use of PGT-A by means of a SNP-based method. Outcomes of more than 1,800 IVF cycles performed at two fertility centers demonstrated that 24-chromosome SNP-based PGT-A and subsequent euploid embryo transfer led to excellent implantation (70%), clinical pregnancy (70%), and live birth (65%) rates. These rates were achieved primarily with the use of SET, and remained high even at advanced maternal ages, in contrast to the age-related decline observed for IVF cycles performed without PGT-A (1, 2). These findings set reliable performance benchmarks for IVF with the use of SNP-based PGT-A, which have not been well defined previously.

Embryo euploidy is an important determinant of sustained implantation and development, and numerous studies have implicated aneuploidy as the primary cause of spontaneous miscarriage (14, 15). Our observation that the proportion of aneuploid TE cells increases with maternal age from approximately one-fourth to two-thirds is consistent with several large studies of the relationships between maternal age and aneuploidy in preimplantation embryos, tested with the use of either SNP-based PGT-A (11, 29) or other CCS methodologies (12). These relationships, which show a rapid increase in aneuploidy and decrease in the probability of obtaining euploid embryos after the age of 35 years, underscore the importance of accurate identification and selective transfer of euploid embryos in women of advanced maternal age undergoing IVF treatment (12).

Consistently with other studies (30, 31), this study demonstrates how PGT-A can help mitigate the negative effects of maternal age on IVF outcomes by allowing the selective transfer of euploid embryos that are more likely to lead to sustained implantation. In addition, it shows that for women in most age categories undergoing IVF treatment implantation, clinical pregnancy, and live birth rates for SET cycles are not significantly different from double-embryo transfer cycles. Taken together, our findings provide evidence that successful IVF outcomes can be achieved without multiple-embryo transfer when transfers are combined with the use of SNP-based PGT-A. We hope that these findings will allay both patient anxiety about the possibility of miscarrying a single transferred embryo (i.e., without a "backup") and physician concern about reduced pregnancy rates (6). Although current embryo transfer guidelines still consider multiple-embryo transfer in women of advanced maternal age to be acceptable (32), a recent survey of fertility specialists showed that most favor SET (18).

There are four commonly used PGT-A methods, each with attendant strengths and weaknesses. The most established method is aCGH, which is considered to be reliable and robust and is supported by numerous clinical studies. qPCR is a faster, cheaper alternative to array-based methods but is not widely used. NGS has rapidly gained popularity because it has a number of advantages, including more sensitive mosaicism detection and mitchondrial DNA analysis, and can be more cost effective than array methods when run in a multiplexed fashion.

SNP-based array methods, such as the one used in this study, have several technical advantages. Unlike aCGH, it can be run with single-gene preimplantation genetic diagnosis, and it allows fingerprinting, which can assist in catching sample swaps. Furthermore, unlike the other methods, SNP-based methods are able to detect a broader spectrum of aneuploidy conditions, including triploidy and haploidy, which would otherwise result in incorrect euploidy determination; therefore, SNP-based PGT-A may be better at identifying euploid embryos than non–SNP-based methods. In a recent study of >18,000 TE biopsies analyzed with the use of this SNP-based method, triploidy and haploidy were observed in ~1.71% and 0.57% of samples, respectively. Because 55.7% of TE biopsies in the present study tested

TABLE 3

Embryo transfers and pregnancy outcomes for all in vitro fertilization cycles in this study, stratified by egg source and maternal age.

		Nondonor (own) eggs					Donor eggs
Outcome	**All ages[a]**	**< 35 y**	**35–37y**	**38–40y**	**41–42y**	**> 42y**	**All ages[b]**
Retrievals, n							
Total	952	264	229	249	144	66	117
Single ET	455	153	123	122	40	17	86
Double ET	99	48	27	21	3	0	17
Euploid embryos available, n	1,756	787	493	357	80	39	589
Embryos thawed, n	813	331	214	197	52	19	175
Transfer procedures, n							
Total	665	256	174	168	49	18	145
Single ET	548	195	144	145	46	18	122
Double ET	116	60	30	23	3	0	23
Embryos transferred, n							
Total	783	318	204	191	52	18	168
Thaw survival rate (%)	96.3	96.1	95.3	97.0	100	94.7	96.0
Average per transfer	1.17	1.24	1.17	1.12	1.06	1.00	1.16
Single ET	548	195	144	145	46	18	122
Double ET	232	120	60	46	6	0	46
Embryos implanted, n							
Total	547	222	153	124	34	14	109
Average per transfer	0.82	0.86	0.87	0.73	0.69	0.78	0.75
Single ET	382	133	110	95	30	14	76
Double ET	163	87	43	29	4	0	33
Implantation rate (%)							
Overall	69.9	69.8	75.0	64.9	65.4	77.8	64.9
Single ET	69.7	68.2	76.4	65.5	65.2	77.8	62.3
Double ET	70.3	72.5	71.7	63.0	66.7	ND	71.7
P value (single vs. double ET)[c]	.95	.74	.79	.89	ND[d]	ND[d]	.60
Clinical pregnancies, n							
Total	472	181	134	112	32	13	94
Single ET	376	131	108	94	30	13	76
Double ET	95	49	26	18	2	0	18
Clinical pregnancy rate (%)							
Overall, per retrieval	49.6	68.6	58.5	45.0	22.2	19.7	80.3
Overall, per ET	70.6	70.4	76.6	65.9	65.3	72.2	64.8
Single ET	68.6	67.2	75.0	64.8	65.2	72.2	62.3
Double ET	81.9	81.7	86.7	78.3	66.7	ND	78.3
P value (single vs. double ET)[c]	.25	.38	.63	.58	ND[d]	ND[d]	.51
Miscarriages, n							
Total	21	6	4	6	5	0	6
Pregnancies with FHT	450	174	128	106	29	13	82
Miscarriage rate (%)	4.7	3.4	3.1	5.7	17.2	0	7.3
Deliveries, n							
Total	429	168	124	100	24	13	76
Single ET	338	119	99	85	22	13	59
Double ET	90	48	25	15	2	0	17
Live birth rate (%)							
Overall, per retrieval	45.1	63.6	54.1	40.2	16.7	19.7	65.0
Overall, per ET	64.5	65.6	71.3	59.5	49.0	72.2	52.4
Single ET	61.7	61.0	68.8	58.6	47.8	72.2	48.4
Double ET	77.6	80.0	83.3	65.2	66.7	ND	73.9
P value (single vs. double ET)[c]	.14	.23	.52	.77	ND[d]	ND[d]	.23
Average ETs per patient needed for a live birth	1.38	1.32	1.23	1.56	1.96	1.39	1.67

Note: ET, embryo transfer; FHT, fetal heart tone; ND, not determined.
[a] Nondonor age range 20–46 y.
[b] Donor age range 20–39 y.
[c] Two-proportion *Z* test; $P<.05$ considered to be statistically significant.
[d] Insufficient double ET data (<5 ET).

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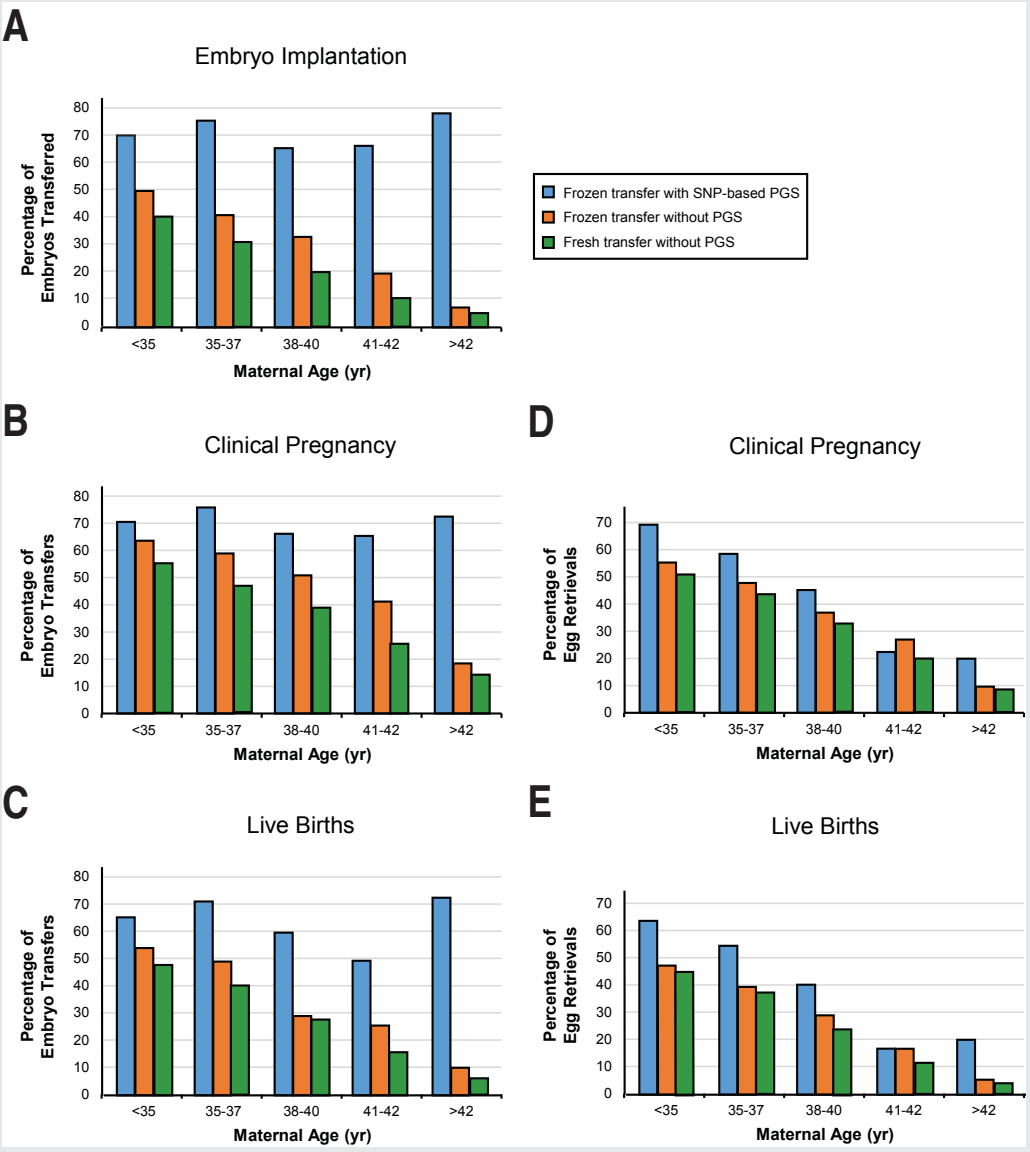
euploid, one would expect that ~4% of embryos that test euploid using a non-SNP method would actually be triploid or haploid (11). Work is ongoing to combine the SNP-based and NGS methods, with the goal of developing a method that combines the advantages of both.

SART data were provided to show maternal-age–related trends seen in non-PGS cases, but implantation, clinical pregnancy, and live birth rates in this study are not directly comparable to data from other studies. This is due to the fact that SART data are pooled from IVF patients at many clinics that use different IVF protocols, as well as to differences in the types, definitions, and units of outcome measures reported (e.g., per patient, per embryo transfer, per cycle). The abundance of different outcome measures not only hinders

FIGURE 1



Comparison of (**A**) implantation rates and (**B, D**) clinical pregnancy and (**C, E**) live birth rates per (**B, C**) embryo transfer or (**D, E**) egg retrieval from nondonor frozen-thawed embryo transfer cycles with the use of single-nucleotide polymorphism (SNP)–based preimplantation genetic testing for aneuploidy (PGT-A; this study; *blue*) along with 2014 U.S. averages for frozen-thawed (*orange*) or fresh (*green*) embryo transfer cycles without PGT-A.

Simon. SNP-based PGT-A improves IVF outcomes. Fertil Steril 2018.

comparisons between studies, but may also give IVF patients unrealistic expectations of success. Because IVF patients in the present study were counseled about their likelihood of having sufficient mature follicles for egg retrieval and having at least one euploid embryo for transfer—with the goal of having a healthy live birth—we reported and compared traditional pregnancy outcomes per embryo transfer and per retrieval, rather than per cycle. However, because more than one-half of all euploid embryos (1,357/2,345; 57.9%; Table 3) in this study remained vitrified, and remain available for thaw and transfer, cumulative pregnancy outcomes per cycle initiated could not yet be determined and are likely to be higher than those reported herein. To improve comparisons, we urge the assisted reproduction community to develop standardized guidelines for reporting outcomes from IVF studies (e.g., implantation rates per transfer, cumulative live birth rates per retrieval, and live birth rates per single- and double-embryo transfer).

This study has two limitations. First, owing to the retrospective nature of the study, not all variables could be controlled. For example, the administration of PGT-A was elective and not randomized, so it is possible that PGT-A was preferentially used in certain cases; the cohort at CRA, with the higher (81%) rate of performing PGT-A, is more likely to be representative of an unselected cohort. Better outcomes were observed at CRA than at PFC; however, with a

lower rate (40% vs. 81%) of patients performing PGT-A, it is possible that the CRA cohort was biased toward patients with better prognosis. Second, the data presented in this study are not sufficient to demonstrate the absolute impact of SNP-based PGT-A on IVF outcomes, because there was no suitable control cohort available. Larger prospective randomized studies are needed to overcome these limitations.

In conclusion, our results show that IVF with the use of 24-chromosome SNP-based PGT-A and subsequent euploid embryo transfer leads to high implantation, clinical pregnancy, and live birth rates, and low miscarriage rates, primarily with SET. We hope that these findings will facilitate discussion and improvement of IVF best practices, including PGT-A-based IVF and SET, and help clinicians to set appropriate expectations for patients undergoing IVF.

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Resultados de embarazos de más de 1800 ciclos de fecundación in vitro mediante polimorfismos de nucleótido único en 24 cromosomas basado en el diagnóstico genético preimplantacional para aneuploidías

Objetivo: Medir los resultados de fecundación in vitro (FIV) con el uso de polimorfismos de nucleótido único en 24 cromosomas (SNP) basado en el estudio genético preimplantacional para aneuploidías (PGT-A) y transferencia de embriones euploides.

Diseño: Retrospectivo.

Lugar: Clínicas y Laboratorios de Fertilidad.

Pacientes: Mujeres de 20 a 46 años de edad sometidas a tratamientos de FIV.

Participantes: Polimorfismo de nucleótido único de 24 cromosomas basado en el estudio genético preimplantacional para aneuploidías de biopsias de embriones de día 5/6.

Medidas de resultado principal: Implantación estratificada por edad materna, embarazo clínico y tasa de recién nacido vivo por embrión transferido; tasas de pérdida/aborto y número de transferencias embrionarias por paciente necesarias para lograr un recién nacido vivo.

Resultados: Se observó una tasa de implantación de 69,9%, una tasa de embarazo clínico por transferencia de 70,6% y una tasa de recién nacido vivo por transferencia de 64,5% en 1621 ciclos congelados de pacientes no donantes con el uso del SNP PGT-A. Además al medir los resultados de SNP PGT-A en ciclos donde hubo transferencias, estos se mantuvieron relativamente constantes a través de todas las edades maternas; al momento de medir los resultados por ciclo iniciado, estos disminuyeron a la vez que la edad materna aumentaba. La tasa de pérdida/aborto fue de aproximadamente 5% en mujeres $\leq$ 40 años de edad. No se hallaron diferencias estadísticamente significativas en los embarazos donde hubo transferencia de un solo embrión (SET) versus aquellos con transferencias de dos embriones y el uso de SNP PGT-A. Un promedio de 1,38 transferencias embrionarias por paciente fueron necesarias para lograr un recién nacido vivo en ciclos de no donantes.

Conclusiones: Nuestros hallazgos muestran que el uso de SNP PGT-A puede mitigar los efectos negativos de la edad materna en los resultados de FIV en los ciclos donde hubo transferencias; y que los resultados de embarazo con ciclos de SET no son significativamente diferentes a aquellos ciclos en los que hubo transferencias de dos embriones, lo que apoya el uso de SET cuando las transferencias embrionarias son combinadas con el uso de SNP PGT-A.