ORIGINAL ARTICLE: ASSISTED REPRODUCTION

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;■:■–■. ©2018 by American Society for Reproductive Medicine.)
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.