Next generation sequencing for preimplantation genetic screening improves pregnancy outcomes compared with array comparative genomic hybridization in single thawed euploid embryo transfer cycles

Jenna Friedenthal, M.D.,[a] Susan M. Maxwell, M.D.,[a] Santiago Munné, Ph.D.,[b] Yael Kramer, M.S.,[a] David H. McCulloh, Ph.D.,[a] Caroline McCaffrey, Ph.D.,[a] and James A. Grifo, M.D., Ph.D.[a]

[a] New York University Langone Fertility Center, New York, New York; and [b] Cooper Genomics, Livingston, New Jersey

Objective: To evaluate whether the use of next generation sequencing (NGS) for preimplantation genetic screening (PGS) in single thawed euploid embryo transfer (STEET) cycles improves pregnancy outcomes compared with array comparative genomic hybridization (aCGH).
Design: Retrospective cohort study.
Setting: Single university-based fertility center.
Patient(s): A total of 916 STEET cycles from January 2014 to December 2016 were identified. Cases included 548 STEET cycles using NGS for PGS and controls included 368 STEET cycles using aCGH for PGS.
Intervention(s): Patients having a STEET after undergoing IVF and PGS with either NGS or aCGH.
Main Outcome Measure(s): Primary outcomes were implantation rate, ongoing pregnancy/live birth rate (OP/LBR), biochemical pregnancy rate (PR), and spontaneous abortion (SAB) rate.
Result(s): The implantation rate was significantly higher in the NGS group compared with the aCGH group (71.6% vs. 64.6%). The OP/LBR was also significantly higher in the NGS group (62% vs. 54.4%), and there were significantly more biochemical pregnancies in the aCGH group compared with the NGS group (15.1% vs. 8.7%). After adjustment for confounding variables with a multiple logistic regression analysis, OP/LBR remained significantly higher in the NGS group. The SAB rate was not significantly different in the NGS group compared with the aCGH group (12.4% vs. 12.7%).
Conclusion(s): Preimplantation genetic screening using NGS significantly improves pregnancy outcomes versus PGS using aCGH in STEET cycles. Next-generation sequencing has the ability to identify and screen for embryos with reduced viability such as mosaic embryos and those with partial aneuploidies or triploidy. Pregnancy outcomes with NGS may be improved due to the exclusion of these abnormal embryos. (Fertil Steril® 2018;109:627–32. ©2017 by American Society for Reproductive Medicine.)
Key Words: Next generation sequencing, array comparative genomic hybridization, preimplantation genetic screening, mosaicism

Discuss: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/28723-25156

Received October 15, 2017; revised November 30, 2017; accepted December 15, 2017; published online March 28, 2018.
J.F has nothing to disclose. S.M.M. has nothing to disclose. S.M. is an employee of CooperGenomics. Y.K. has nothing to disclose. D.H.M. reports compensation from ReproART: Georgian-American Center for Reproductive Medicine, Tbilisi, Georgia, Biogenetics Corporation, Mountainside, NJ, and Sperm and Embryo Bank of New York, New York, NY, outside of the submitted work. C.M. has nothing to disclose. J.A.G. has nothing to disclose.
Reprint requests: Jenna Friedenthal, M.D., Department of Obstetrics and Gynecology, New York University Langone Fertility Center, New York, New York 10016 (E-mail: Jenna.friedenthal@nyumc.org).

Fertility and Sterility® Vol. 109, No. 4, April 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2017.12.017

Chromosomal abnormalities are the most frequent cause of first trimester pregnancy losses, and account for >50% of spontaneous abortions (1–3). In patients with advanced maternal age or recurrent pregnancy loss, IVF with preimplantation genetic screening (PGS) was developed as a means to identify and exclude chromosomally abnormal embryos, thereby increasing implantation rates and decreasing spontaneous abortion (SAB) rates. Various genetic platforms are available for comprehensive chromosomal screening for PGS. The most used platforms are array comparative genomic hybridization (aCGH) (4–6), single nucleotide polymorphism array (7, 8), quantitative polymerase chain reaction (PCR) (9–11), and next generation sequencing (NGS) (12–14).

Next generation sequencing is the newest platform for PGS, which performs high throughput and high resolution sequencing by synthesis. Table 1 summarizes the detection capability of the different platforms according to the published literature. All platforms can assess aneuploidy of full chromosomes with low error rates, but not all platforms can reliably detect unbalanced translocations, segmental aneuploidies, polyploidy, or mosaicism. Mosaicism is defined as the presence of two or more populations of cells, each with different genotypes, within the same embryo and results from mitotic errors occurring after fertilization. Next generation sequencing has gained in popularity due to its ability to identify unbalanced translocations, segmental aneuploidies, some triploidies (20), and lower levels of mosaicism than other techniques (17, 21). This may be clinically important because mosaic embryos seem to have impaired viability and a decreased potential to result in a live birth.

Maxwell et al. (20) re-examined embryos that were diagnosed as euploid by aCGH and resulted in miscarriage. Using NGS, they found that 31.6% of these miscarriages were from mosaic embryos and 5.2% were from triploid embryos. Array CGH was unable to detect these chromosomal abnormalities in whole genome amplified DNA from the same trophectoderm biopsy samples. In a multicenter study, Munné et al. (17) found that mosaic embryos diagnosed with NGS had significantly lower implantation rates than euploid embryos. Complex mosaic embryos and mosaic embryos with 40%–80% abnormal cells also had lower ongoing implantation rates than other types of mosaic embryos. It is estimated that 20%–30% of blastocysts undergoing PGS with NGS are diagnosed as mosaic.

The purpose of this study was to determine whether the use of NGS, with its ability to exclude more mosaic embryos, improves pregnancy outcomes compared with aCGH when performing single thawed euploid ETs (STEET). We hypothesized that the clinical implementation of NGS would increase implantation and ongoing pregnancy rates (PRs) and decrease SAB rates among patients undergoing IVF with PGS.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board at New York University School of Medicine (NYU IRB#13-00389). This is a retrospective cohort study of all STEET cycles from January 2014 to December 2016 at a single large university-based fertility center. Cases included all STEET cycles using NGS for PGS and controls included all STEET cycles using aCGH for PGS. The primary outcomes were implantation rate, SAB rate, biochemical PR, and ongoing PR/live birth rate. The implantation rate was calculated as the number of gestational sacs visualized on transvaginal ultrasound divided by the total number of embryos transferred. The SAB rate was defined as a pregnancy failure after a previously documented gestational sac on transvaginal ultrasound divided by the total number of clinical pregnancies. Biochemical pregnancies were defined as a positive hCG level on cycle days 28–30 $\geq$5 mIU/mL followed by declining hCG levels before the development of a gestational sac on transvaginal ultrasound. The biochemical PR was calculated as the number of biochemical pregnancies per ET with a subsequent positive hCG. The ongoing PR/live birth rate was defined as the number of ongoing pregnancies after the presence of a fetal pole with fetal heart tones and/or live births divided by the total number of embryos transferred. Monozygotic twins resulting from the transfer of one embryo were counted as one implantation and one ongoing pregnancy or live birth.

At our center, all physicians used aCGH in 2014. Starting in January of 2015, NGS became available for PGS; therefore, two-thirds of our physicians elected to use NGS exclusively for their PGS cycles. One-third of our physicians continued to use aCGH for all of their PGS cycles in 2015. As of 2016, all physicians in the practice used NGS for PGS. We performed a subanalysis comparing only PGS cycles in 2015 in an attempt to control for changes in laboratory practices over time.

TABLE 1

A comparison of current preimplantation genetic screening platforms for comprehensive chromosomal screening.

Characteristics	qPCR	aCGH	SNP array	High resolution NGS
Total independent data signals[a] (reads per sample)	96	2,700	32,000	700,000
Resolution in million megabytes	20	6	6	3
Misdiagnosis of aneuploidies (4, 9, 12, 13, 15)	1%	2%	2%	0
Unbalanced translocations (16)	No	Yes	Yes	Yes
Partial aneuploidies	No	Yes	Yes	Yes
Polyploidy	No	No	Yes	Yes
Percent mosaicism detectable (17, 18, 19)	No	40%–60%	No	20%–80%

Note: aCGH = array comparative genomic hybridization; NGS = next generation sequencing; qPCR = quantitative polymerase chain reaction; SNP = single nucleotide polymorphism.
[a] Number of reads per run × number of samples per run × percent of reads lost = number of reads per sample.

Friedenthal. NGS increases ongoing PRs. Fertil Steril 2017.