Changing ovarian stimulation parameters in a subsequent cycle does not increase the number of euploid embryos

Brooke Hodes-Wertz, M.D., M.P.H., David H. McCulloh, Ph.D., Alan S. Berkeley, M.D., and Jamie A. Grifo, M.D., Ph.D.

New York University Fertility Center, Langone Medical Center, New York University, New York, New York

Objective: To compare the euploidy outcome in patients that underwent 2 ovarian stimulation cycles with trophectoderm biopsy.
Design: Retrospective repeated-measures cohort study.
Setting: University-based fertility center.
Patient(s): A total of 116 patients, from 2011 through 2013, that underwent 2 ovarian stimulation cycles followed by trophectoderm biopsy with array comparative genomic hybridization.
Intervention(s): Days of stimulation, average diameter of the 2 lead follicles on day of trigger, dose of gonadotropins, type of cycle (gonadotropin-releasing hormone [GnRH] antagonist, GnRH-antagonist plus clomiphene citrate [CC], microdose GnRH agonist).
Main Outcome Measure(s): Number of euploid embryos.
Result(s): Patients were analyzed based on whether they had ≥ 1 euploid embryos in their first cycle vs. none. There was no increase in the number of euploid embryos with more days of stimulation or increases in the dose of gonadotropins in either group. Significantly more euploid embryos were seen in patients who had no euploid embryo(s) in the first cycle (Group 0) that had CC added to a GnRH-antagonist cycle (1.11 more euploid embryos) or were triggered when follicle sizes were 2 mm larger (0.40 euploid embryos), but these increases were not significant compared with a control group. Patients with euploid embryo(s) in the first cycle (Group 1) had significantly more euploid embryos when daily dose was increased by 75–149 international units, but this relationship was not significant compared with a control group with no increase in daily dose.
Conclusion(s): No specific intervention increased the number of euploid embryos within the same patient any more than simply repeating a similar stimulation cycle. An attempt was made to control for interpatient variability, but individual patients have considerable intercycle variability. (Fertil Steril® 2015;103:947–53. ©2015 by American Society for Reproductive Medicine.)
Key Words: Euploidy, controlled ovarian stimulation, array comparative genomic hybridization

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/hodeswertzb-ovarian-stimulation-euploidy/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Since the inception of in vitro fertilization (IVF), the ultimate goal has been to provide patients with a healthy child. To reach this goal, studies have typically measured success by pregnancy or live-birth rates. However, numerous maternal, embryonic, fetal, and placental variables make adequate assessment of treatment effects difficult. The ploidy status of embryos can now provide a new end-point to gauge interventions that avoids the complexity of controlling the many variables of implantation and pregnancy. Euploidy, a prerequisite to a healthy live birth, can now be accurately and safely diagnosed with trophectoderm (TE) biopsy (1) and methods that assess all 24 chromosomes, such as array comparative genomic hybridization (aCGH) (2, 3), single-nucleotide polymorphism microarrays (4, 5), and quantitative polymerase chain reaction (6). In the past, in an attempt to increase live-birth rates, the aim of ovarian stimulation has been to maximize the number

Received November 7, 2014; revised December 4, 2014; accepted December 30, 2014; published online February 20, 2015.
B.H.-W. has nothing to disclose. D.H.M. has nothing to disclose. A.S.B. has nothing to disclose. J.A.G. has nothing to disclose.
Reprint requests: Brooke Hodes-Wertz, M.D., M.P.H., New York University Fertility Center, Langone Medical Center, 660 First Ave, 5th Floor, New York, New York 10016 (E-mail: hodesb01@med.nyu.edu).

Fertility and Sterility® Vol. 103, No. 4, April 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.12.126

of oocytes retrieved, to combat the attrition that occurs with IVF. Now that ploidy (one important component of embryo quality) can be precisely assessed, the focus can shift to optimizing the number of chromosomally normal embryos. In addition, in our center, after TE biopsy, embryos are vitrified, and euploid embryo(s) are transferred in a subsequent thaw cycle. Therefore, we can concentrate solely on effects of ovarian stimulation on euploidy, without concern for endometrial effects, which need to be monitored in a fresh cycle.

A quest has long been underway to determine the best protocol for ovarian stimulation, with a heavy focus on gonadotropin dose, length of stimulation, and size of the follicles at the time of trigger injection (7–10). In addition, a debate has unfolded over whether these stimulation parameters are related to euploidy (11–23).

In our initial attempts to assess this relationship, we performed a Poisson regression on 363 patients that underwent ovarian stimulation followed by TE biopsy and aCGH (24). The following variables were assessed in an attempt to fit the regression model: stimulation parameters (gonadotropin type, starting dose, average daily dose, total dose, gonadotropin-releasing hormone [GnRH] antagonist or GnRH agonist [GnRH-a], type of trigger, use of other drugs) and response parameters (estradiol levels on the day of human chorionic gonadotropin [hCG] and the day after, number of oocytes, number of mature oocytes, number of fertilized oocytes, size of lead follicles).

Of these, only 3 variables were found to be associated with the number of euploid embryos. Not surprisingly, higher ages were associated with fewer euploid embryos. However, more gonadotropins was associated with a decrease in euploid embryos, whereas increasing the amount of mature oocytes was associated with an increase of euploid embryos. A remaining question was whether increasing the dose was deleterious or rather that these trends were a reflection of a lower frequency of euploidy when patients respond poorly and require more gonadotropins.

To avoid this dilemma, and control for interpatient variability, we reviewed ovarian stimulation cycles and euploidy outcomes within the same patient, allowing each patient to serve as her own control. The objective of this study was to determine which ovarian stimulation parameters led to more euploid embryos, by examining the differences in ovarian stimulation and euploidy in patients that were stimulated and underwent TE biopsy more than once.

MATERIALS AND METHODS

This retrospective review included all patients that underwent >1 ovarian stimulation cycle followed by TE-aCGH, from 2011 through 2013. Any biopsies done on previously frozen, thawed blastocysts, or embryos from thawed oocytes, were excluded. In addition, patients that underwent TE biopsy for translocation detection were excluded. The first 2 TE-aCGH cycles were included for analysis.

Each patient's ovarian stimulation was individualized to achieve adequate numbers of mature oocytes at retrieval. On average, when ≥ 2 lead follicles had a mean diameter of 17–19 mm, a trigger injection (hCG, GnRH-a, or a combination of the 2) was administered. Oocyte retrieval, fertilization, embryo culture, laser hatching on day 3, TE biopsy, and vitrification were all performed as described previously by Grifo et al. (25). Intracytoplasmic sperm injection was performed only when indicated for severe male factor or history of poor fertilization, or for single-gene testing. A hole was made in the zona pellucida on day 3 of embryo development using a Saturn Active Laser System using Cronus Embryo Analysis (Research Instruments), to allow hatching.

After zona incision, embryos were cultured to day 5, and embryos not suitable for biopsy on day 5 were cultured to day 6 (or, very rarely, to day 7). On the designated day (day 5, 6, or 7), TE biopsy was performed, and a piece of the extruded TE was isolated and cut using a laser. Embryos were cryopreserved using vitrification. Once all biopsies were performed, most specimens were transported to Reprogenetics for preimplantation genetic screening analysis using aCGH. Specimens for 3 patients were sent to Natera for single-gene testing and aCGH.

The following stimulation interventions were assessed: days of stimulation, amount of gonadotropins given, average size of the 2 lead follicles on the day of trigger injection, and type of stimulation cycle (GnRH-antagonist cycle, GnRH-antagonist cycle with clomiphene citrate [CC], or microdose GnRH-a). Comparsions were made of primary and secondary outcomes between the patient's first and second TE cycle. The primary outcome measure was number of euploid embryos. Secondary outcome measures included: number of oocytes retrieved, number of blastocysts for biopsy on day 5 and total number of embryos for biopsy, and percentage of euploid biopsied embryos (calculated as the number of euploid embryos divided by the total number of embryos biopsied).

When a patient had a change in any variable(s) (days of stimulation, amount of gonadotropins given, average size of the two lead follicles on day of trigger injection or type of stimulation cycle), those cycles were considered intervention cycles, and the primary and secondary outcomes of those cycles were compared to a "control" or nonintervention group of patients that had no change in that variable between cycles. For example, outcomes in patients that had an increase in gonadotropin dose were compared with outcomes in patients that did not have a change in gonadotropin dose. In addition, baseline cycle characteristics, such as age, total amount of gonadotropins used, the number of days of stimulation, and the average size of the lead follicles, were compared for cycles with vs. without the intervention.

All data were analyzed using SPSS, Version 20.0 (SPSS Inc). The Shapiro-Wilk test was used to assess normality of the differences between the pairs (for baseline characteristics, and primary and secondary outcomes). Based on these results, either a paired t test (normally distributed) or Wilcoxon's signed rank test (non-normal distribution) was used to compare the differences between the 2 cycles. In addition, the change in outcome (the mean difference) was compared to the amount of change between the first and second cycle of the same outcome in a group of patients that had no change in the intervention, using an independent t test or analysis of variance, as indicated. Correlations between the change in number of euploid embryos and change in dose, days of