ORIGINAL ARTICLE: EARLY PREGNANCY

Higher rates of aneuploidy in blastocysts and higher risk of no embryo transfer in recurrent pregnancy loss patients with diminished ovarian reserve undergoing in vitro fertilization

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Objective: To study the prediction of aneuploidy rate in blastocysts from patients with recurrent pregnancy loss (RPL) on the basis of ovarian reserve testing.
Design: Prospective cohort analysis.
Setting: Private, university-affiliated fertility clinic.
Patient(s): A total of 239 patients with RPL, defined as two or more clinical miscarriages, were screened for inclusion. One hundred two (102) cycles in patients with unexplained RPL resulted in at least one euploid embryo transferred. Outcomes were compared by ovarian reserve test results, with diminished ovarian reserve (DOR) defined as a cycle day 3 FSH >10 IU/mL and/or antimüllerian hormone <1 ng/mL.
Intervention(s): In vitro fertilization with blastocyst biopsy and aneuploidy screening of all 23 chromosome pairs.
Main Outcome Measure(s): Rate of aneuploidy in blastocysts and incidence of IVF cycles with no transfer owing to no euploid blasts.
Result(s): Patients with DOR had a higher percentage of aneuploid blastocysts (57% vs 49%) and a higher incidence of no euploid embryos to transfer (25% vs 13%). The higher rate of aneuploidy in blastocysts was most significant in patients aged <38 years (67% vs 53%). Implantation rates after transfer of euploid blastocysts were similar (61% compared with 59%), and miscarriage rates were low (14% and 10%).
Conclusion(s): Unexplained RPL patients with DOR have a higher percentage of aneuploid blastocysts and risk of no euploid embryo to transfer compared with unexplained RPL patients with normal ovarian reserve testing. The difference is most significant in patients aged <38 years. Patients with RPL and DOR with euploid embryo transferred had similar outcomes compared with patients with RPL and normal ovarian reserve testing. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, diminished ovarian reserve, in vitro fertilization, preimplantation genetic screening, recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is a clinically frustrating and emotionally charged challenge for patients and providers alike. In 2013 the American Society of Reproductive Medicine (ASRM) stated that RPL is a disease distinct from infertility, which is defined by two or more clinically recognized pregnancy losses (1). An evaluation of a couple with RPL, which includes parental karyotypes, uterine cavity evaluation, hormonal testing, and screening for antiphospholipid syndrome, will find a cause for RPL in less than 50% of cases (2, 3).

A high incidence of aneuploidy in products of conception from

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first-trimester losses can potentially explain repeated miscarriage in patients with otherwise unexplained RPL (2, 4, 5). The incidence of aneuploidy increases with maternal age (6) and number of previous miscarriages (7). In vitro fertilization with chromosomal screening (CS) of embryos for aneuploidy has been proposed as a treatment option for women with RPL (8).

Katz-Jaffe et al. (9) showed a higher incidence of aneuploid blasts in patients with diminished ovarian reserve (DOR), and other studies have shown a higher than expected incidence of DOR in RPL patients (10, 11). No published studies have shown what patients with RPL and DOR, compared with other patients with unexplained RPL, might expect should they undergo an IVF cycle.

The objective of this study was to examine the role of ovarian reserve testing in predicting rate of aneuploidy and the risk of having no euploid embryo to transfer in patients with unexplained RPL who choose IVF with CS as a treatment option.

MATERIALS AND METHODS

This study is a prospective cohort study of IVF outcomes in unexplained RPL patients who underwent IVF with preimplantation CS from January 1, 2011 to March 31, 2015 at a private, university-affiliated fertility center in Seattle, Washington. Institutional review board approval was obtained for the project.

Clinical miscarriage was defined as pregnancy loss before 20 weeks' gestation after confirmation with ultrasound (gestational sac or fetal pole) or products of conception diagnosed histologically by patient report or medical records. Patients presenting for RPL were offered an evaluation if they had a history of two or more clinical miscarriages. Unexplained RPL was defined as patients without uterine cavity defects, balanced translocation, antiphospholipid syndrome, or endocrine disorders (12).

Patients underwent ovarian reserve testing with both serum antimüllerian hormone (AMH) levels and cycle day 2 or 3 serum FSH and E_2, although this is not included in ASRM guidelines for evaluation of RPL (12). Follicle-stimulating hormone was measured by electrochemiluminescent immunoassay, and AMH was measured with an ELISA (Gen II ELISA reference A79765) (13, 14). A patient was considered to have DOR if their FSH was $\geq$10 mIU/mL and/or their AMH level was $<$1 ng/mL (13, 15). Antral follicle count was not used as a measure of ovarian reserve owing to its subjective nature and the previous description of the close correlation of antral follicle count and serum AMH (15). Patients were excluded if they had uncorrectable uterine cavity defects, antiphospholipid syndrome, or balanced translocations.

Standard protocols for controlled ovarian hyperstimulation were used. Protocol and dose of medication was determined for individual patients by their provider, with the decision based on patient's age, history, and ovarian reserve testing. Protocols for normal ovarian reserve patients included either the use of a mid-luteal phase GnRH agonist for 7 days before initiation of gonadotropin stimulation or a GnRH antagonist initiated after gonadotropin start when the lead follicle reached 12–14 mm, using an average daily gonadotropin dose of 300 IU. Protocols for poor ovarian reserve patients included either an antagonist protocol or microdose flare protocol with a maximum daily gonadotropin dose of 450 IU. Human chorionic gonadotropin (10,000 IU) was administered when two lead follicles reached a mean diameter of 18–20 mm. A dual trigger of leuprolide acetate with 1,500 IU of hCG was used if patients were considered at risk for ovarian hyperstimulation syndrome (E_2 level $>$4,000 pg/mL, 20+ mature follicles, or were symptomatic). The transvaginal ultrasound-guided oocyte aspiration was performed 35 hours after the patient received the trigger shot.

In vitro fertilization, embryo culture, and blastocyst biopsy techniques were performed in a manner previously described by others (16). Intracytoplasmic sperm injection was performed in all cases. Trophectoderm biopsy was performed on all expanding or fully expanded blastocysts on postretrieval day 5 or 6, depending on the rate at which individual embryos reached this developmental stage. All embryos underwent vitrification after trophectoderm biopsy. Each trophectoderm biopsy sample underwent CS analysis at a commercial preimplantation genetic diagnosis laboratory (Genesis Genetics, false-positive and false-negative rates of 2%, respectively). Metaphase comparative genomic hybridization with microarray analysis was used.

Only euploid embryos were warmed and subsequently transferred after adequate endometrial preparation and luteal support. Frozen embryo transfer protocols included either a programmed cycle with oral contraceptive pill preparation and estrogen supplement in the form of E_2 patches or IM estrogen valerate, or a natural protocol in which patients were monitored through their own menstrual cycle. Luteal support in the form of IM P or vaginal suppository was begun after the endometrial lining reached 8 mm and a serum E_2 level of $\geq$150 pg/mL was observed. Embryo transfer occurred on the sixth day of P supplementation in a programmed cycle and on the sixth day after hCG administration in a natural cycle.

All patients had a normal uterine cavity evaluation before transfer. Embryo transfer was performed under transabdominal ultrasound guidance after embryo survival was confirmed after warming. Either one or two embryos were transferred, with the majority of patients (93%) having a single embryo transferred. A serum pregnancy test was obtained 10 days after ET, and a transvaginal ultrasound was performed at approximately 6 and 8 weeks' gestation. Implantation rate was defined as the number of gestational sacs noted on ultrasound examination per number of embryos transferred. Data were analyzed with Student t tests and χ^2 tests as appropriate, and a P value $<$.05 was considered to be statistically significant.

RESULTS

All patients presenting to our clinic from January 1, 2011 to March 31, 2015 were screened for inclusion. Patients with unexplained RPL who opted for IVF with preimplantation CS as a treatment option and had embryos screened for aneuploidy were included in the analysis. A total of 239 patients had two or more clinical miscarriages and received the ASRM evaluation for RPL (12). Of these, 179 patients had no explanation