Aneuploidy rates in embryos from women with prematurely declining ovarian function: a pilot study

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Objective: Prematurely declining ovarian function (PDOF) affects approximately 10% of infertile females, and has been suggested to represent a shift of the normal ovarian aging curve toward younger age. Whether women with PDOF demonstrate an increased level of aneuploidy in their embryos, based on increasing aneuploidy rates with advancing female age, is unknown, and was the subject of this study.
Design: Retrospective, case-control study.
Setting: Academically affiliated private IVF center.
Patient(s): Twenty women with PDOF, and 20 age-matched controls with age-appropriate ovarian function (AAOF), underwent IVF cycles and preimplantation genetic diagnosis (PGD) by fluorescence in situ hybridization for chromosomes X, Y, 13, 16, 18, 21, and 22 on day 3 after fertilization, and ET on day 5.
Intervention(s): None.
Mean Outcome Measure(s): Oocyte and embryo numbers, embryonic aneuploidy rates, pregnancies, and miscarriages.
Result(s): Pregnancy rates (PRs) after initial fresh ET did not differ between patients with PDOF and those with AAOF. Among a total of 258 embryos analyzed by PGD, aneuploidy rates in PDOF and AAOF cycles were 52.6% and 52.2%, respectively. A trend toward lower ongoing PRs was noted in patients with PDOF (21% versus 41%), primarily attributable to higher clinical miscarriage rates (50% versus 13%) after detection of fetal heart motion.
Conclusion(s): In this pilot study, the observation that PDOF is not characterized by an increased aneuploidy rate (controlled for age) suggests that PDOF does not represent a simple shift of the physiologically declining ovarian function curve toward younger age. This observation, indeed, suggests that the underlying pathophysiology of PDOF may vary from that of AAOF. (Fertil Steril® 2007;88:90–4. ©2007 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy, IVF, miscarriage, PGD, poor responder, prematurely declining ovarian function, premature ovarian failure

Prematurely declining ovarian function (PDOF) occurs in approximately 10% of infertile females, and has been suggested to occur at an even higher prevalence in women with so-called *unexplained* infertility (1, 2). The diagnosis of PDOF should be suspected if women, at an inappropriately young age, already demonstrate evidence of diminished ovarian reserve, which can manifest itself either in abnormal ovarian function tests and/or through the presence of significant resistance to ovarian stimulation with gonadotropins (3–6). However, such clinical findings are also characteristic of the ovary that is aging physiologically and according to age-appropriate ovarian function (AAOF) (7–9). Therefore, some investigators recently suggested that PDOF may simply represent a shift of the normal ovarian aging curve toward younger age (1, 10).

After IVF, women with PDOF have a lower probability of pregnancy than expected for their age group (11–14). A number of recent reports suggested, however, that their prognosis may not have to be as poor as has been assumed. Such evidence first came from reports that demonstrated that elevated baseline FSH levels in young women had less negative predictive value for IVF cycle outcomes than in older women (15–17). We, as well as others, previously reported that pregnancy rates (PRs) after initial, fresh IVF cycles in young women with PDOF approached those of age-matched patients with AAOF, if PDOF patients received ovarian stimulation which was based on the women's ovarian function instead of chronological age (10, 18).

These divergances from the expected IVF-cycle outcomes for "older" ovaries raise the question of whether PDOF

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represents a shift of the normal, physiological ovarian aging curve toward younger age, or rather are the result of a distinct, and different, pathophysiology from AAOF.

Assuming that PDOF reflects accelerated ovarian aging, one would not only expect the clinical features of diminishing ovarian reserve, such as lower oocyte numbers and abnormal ovarian function tests (19, 20), in PDOF patients, but also the well-described increase in aneuploidy rate with advancing female age (21–25). Whether aneuploidy rates are, in fact, increased in women with PDOF is unknown, and is the subject of the present case-control study.

MATERIALS AND METHODS

Using our center's computerized database, we identified 20 women with PDOF who had undergone preimplantation genetic diagnosis (PGD) in association with one IVF cycle. The women ranged in age between 27–40 years, and represented consecutive cycles with a PDOF diagnosis that underwent IVF and PGD at our program between August 2003 and December 2004 (the study group). These patients and their cycles were matched with 20 women of identical ages, and with proven AAOF, who had also undergone IVF and PGD during the same time period. These patients were considered the control group.

We considered patients to have a diagnosis of PDOF if they had a history of ovarian resistance or, in a minority of cases, elevated baseline FSH levels (day 2 and 3 FSH levels ≥10 mU/mL). Ovarian resistance was diagnosed if women with age-appropriate ovarian stimulation in the previous cycle (up to age 35 years, 225–300 IU of gonadotropins per day; age 36–40 years, 300 IU per day) produced ≤7 oocytes under age 30, ≤5 between ages 30–35 years, and ≤3 between ages 36–40 years.

In contrast, AAOF was considered to be present if patients produced, with age-appropriate ovarian stimulation, normal age-appropriate oocyte numbers, defined as ≥8 oocytes under age 30 years, ≥6 oocytes between ages 30–35 years, and ≥4 oocytes between ages 36–40 years during this cycle, and showed baseline FSH levels <10 mU/mL.

Indications for fertility treatment were tubal and male factor, ovulatory dysfunction, and advanced female age. In patients with PDOF, ovarian hyperstimulation was performed with a microdose agonist protocol. Starting on cycle day 2, 40 μg of leuprolide acetate (Tap Pharmaceuticals, Lake Forest, IL) were administered SC twice daily, followed by a daily dose of either 450–600 IU of recombinant FSH (recFSH) (Ares-Serono, Geneva, Switzerland), or 300–450 IU of recFSH and 150 IU of hMG (Ares-Serono), after 3 days of agonist treatment. In addition, one patient received 75 mg of dehydroepiandrostenedione per day. Cycles with AAOF were treated with long agonist protocols, starting on day 21 of the previous cycle, with 500 μg of leuprolide acetate (Tap Pharmaceuticals) twice daily (BID), followed by a daily dose of 225–300 IU of recFSH (Ares-Serono), or 150 IU of recFSH and 150 IU of hMG (Ares-Serono). When the lead follicle diameter reached 18–20 mm, follicular maturation was triggered with an injection of 10,000 IU of hCG, with oocyte retrieval 34 hours later.

Patients with PDOF and AAOF mainly underwent PGD for gender selection. However, two couples in each group had PGD because of advanced female age. Preimplantation genetic diagnosis was performed by fluorescence in situ hybridization for chromosomes X, Y, 13, 16, 18, 21, and 22 on day 3 after fertilization, and ET at blastocyst stage, on day 5 after fertilization.

All patients were represented by only one cycle, and the statistical analysis was performed using SPSS for Windows, standard version 10.0.7 (SPSS, Inc., Chicago, IL). To assess the statistical power of our study in terms of clinical significance, we addressed the following considerations. The association between accelerated follicular loss and increasing aneuploidy is widely acknowledged (1, 22), and is believed to begin at approximately age 37 years (26). Eventually, menopause occurs before the follicular supply is depleted, currently at a mean age of 51 years (7). Premature ovarian failure (POF) is defined by the occurrence of menopause at ≤40 years of age (27), i.e., 11 years ahead of the current average age at menopause (7). Assuming that PDOF represents a shift of the ovarian aging curve toward younger age, one would expect the beginning of accelerated follicular loss, and increasing aneuploidy rates, in patients with PDOF at approximately age 26 years instead of age 37 years (1). If the hypothesis of PDOF as a process of accelerated ovarian aging is correct, then the patients with PDOF in our study group, who were aged 27–40 years, should experience increased aneuploidy rates, compared with controls with AAOF.

Based on published data on oocyte donors (24), we estimated that ≤50% of embryos obtained after ovarian stimulation from patients ≤35 years old would be aneuploid. We further estimated, in accordance with data presented by Taranissi et al. (25), that approximately 75% of embryos obtained from patients aged >40 years would be aneuploid. These results correspond to our center's own PGD data, which revealed aneuploidy rates of 45% (at ≤35 years of age), comparable to our study and control groups, and aneuploidy rates of 67% (>40 years of age), irrespective of oocyte numbers.

Therefore, we also tested the distribution of observed aneuploid and euploid embryos in patients with AAOF and those with PDOF against the expected distribution of approximately 50% and 75% aneuploidy, respectively, assuming that patients with PDOF were actually performing like older women. The rather low patient number cannot completely rule out a type II error. However, the fact that patients were matched for age, and that age is known to be the most important influencing factor for embryonic aneuploidy, reduced the likelihood of differences anticipated by power analyses. The significance of the difference in distribution