Advanced maternal age patients benefit from preimplantation genetic diagnosis of aneuploidy

Chromosome abnormalities in human embryos may result in implantation failure or miscarriage. These abnormalities are common, and their incidence increases with advancing maternal age, from approximately 40% in fertile egg donors to 80% in patients 41 to 42 years old (1). Preimplantation genetic diagnosis of aneuploidy (PGD-A) is used as a selection tool for euploid embryos with potential to implant and reach term. That chromosome abnormalities are a major cause of embryo loss with advancing maternal age is demonstrated by the observation that once a euploid embryo is transferred to the uterus, it seems to have the same chance of implanting irrespective of maternal age (2). Preimplantation genetic diagnosis of aneuploidy has evolved from its first iteration using day-3 biopsy and testing for a limited number of chromosomes by fluorescence in situ hybridization, to blastocyst biopsy and comprehensive 24-chromosome screening (CCS) techniques including array comparative genomic hybridization, quantitative polymerase chain reaction, single-nucleotide polymorphism array, or next-generation sequencing.

Three previous randomized clinical trials (RCT) using the latter technologies have focused on young or good-prognosis patients and have found improvements in ongoing pregnancy rates, but the trials were underpowered. The study by Rubio et al. (3) in this issue of *Fertility and Sterility* is the first RCT involving day-3 blastomere biopsy, and PGD-A by CCS, targeting solely patients of advanced maternal age. Array comparative genomic hybridization in this multicenter study produced an acceptable no-call rate of 2.8%. On average, the study patients had five day-3 embryos, of which 62% developed to blastocyst. Compared with previous RCTs, the present study patients would be placed in the category of poor prognosis, with 78% chromosomally abnormal embryos (4). Thus, on average they produced a single euploid day-3 embryo or 0.6 euploid blastocysts per patient.

In this challenging group, with limited choice of embryos for transfer, the study shows that PGD-A significantly improved implantation, reduced miscarriage, and improved delivery rates both per transfer and per intention to treat cycle. These results offset a sharp decrease in the incidence of embryo transfer after PGD-A compared with the control arm. The design of this study offers a clear advantage over other PGD-A trials because all embryos were biopsied on the same day (day 3), whereas in blastocyst biopsy studies, data evaluation is complicated owing to biopsy being performed on both day 5 and day 6. The authors also offered a cost-effectiveness analysis, showing that in their system the costs for a single live birth were higher with PGS than without. However, they also argued that incorporating blastocyst biopsy and next-generation sequencing as standard of care could reduce these costs by 10% both in Europe and in the United States.

The cumulative delivery rate per patient was not significantly different, but an advantage of PGD-A would not be expected if all euploid embryos were to be available for transfer. Indeed, the opposite would occur, that is, the control group would show higher cumulative delivery rates if the euploid embryo pool were to be reduced either by biopsy/vitrification damage or misdiagnosis as aneuploid. From a "cumulative delivery rate" point of view PGD-A is applied to reduce the risk of miscarriage and its associated psychological and physical trauma, as well as to reduce time to pregnancy. In the present study these objectives were achieved, that is, pregnancy loss rates were reduced dramatically, and time to achieve an ongoing pregnancy was reduced by half.

The development of PGD-A over the past 10 years was predicated on two premises, [1] that 24-chromosome testing would lead to decreased error and reduced no-call rates ($\leq$2%), and [2] that the potentially negative effects of the biopsy procedure itself would be avoided if blastocysts rather than cleavage stage embryos were to be biopsied. The study of Rubio et al. brings the second premise into question because they performed the biopsy on day 3 of development.

Although current literature suggests that blastocyst biopsy may be safer than blastomere biopsy, it is likely that any cell biopsy has an effect on the developing embryo. There is conflicting evidence from day-3 biopsy studies. Most PGD-A RCTs involving day-3 biopsy and fluorescence in situ hybridization were performed by laboratories with limited experience in day-3 biopsy, whereas centers reporting improved results had extensive experience in biopsy but failed to produce level 1 evidence. Rubio et al.'s study was performed at IVI, which is a network of centers with more than 15 years of experience in day-3 biopsy. Thus these investigators show that in skilled hands, pregnancy results can be improved even with day-3 biopsy (5). This is not to say that clinics should offer day-3 biopsy and CCS in lieu of trophectoderm biopsy. The combined evidence still suggests that blastocyst biopsy may be relatively easier to master, whereas day-3 biopsy requires a higher skill level, including years of experience performing the procedure. In our opinion, as suggested by the authors, blastocyst biopsy should be the method of choice for most fertility centers.

Like previous randomized PGD-A studies, there are limitations in the study design. Clinical and laboratory staff monitoring the RCT cycles were not blinded to the allocation of patients, and neither was allocation concealment applied to the patients. The study is underpowered, and although two culture media systems were used, data were not evaluated according to the culture system. Most exclusions after randomization were due to low number of mature oocytes (metaphase II), especially in the control group, which could potentially bias the final analysis. Some patients with previous miscarriages were excluded, but it is unclear whether those miscarriages were due to chromosomal abnormalities, as the exclusion criteria require. Similarly, recurrent implantation failure patients were excluded, but that was not an exclusion criterion.

It is important to note that the control group in this study had pregnancy outcomes within the expected range, at least

according to the national US data published by the Society for Assisted Reproductive Technology (SART) for the years 2013 and 2014. The miscarriage rate in the control group was 39%; this rate is comparable to the 26% and 40% miscarriage rate for SART age groups 38–40 years and 41–42 years, respectively. Implantation rates in the SART report were 19.3% and 10.2% for the same age groups, as compared with 27% for the study control group. The proportion of fresh cycles resulting in live birth was 22% and 12% in the SART report, as compared with 24% in the study control group. Overall, results for the control group were within the expected range, suggesting that this group is generally representative. By contrast, some RCTs seem to have a "magic" control group that is unexpectedly suitable for the point the authors wish to make, but far from what would be expected from an average group of patients.

Single embryo transfer could have further enhanced this study. The authors have shown that the risk of pregnancy loss is dramatically reduced by PGD-A, but they did not tackle the risk of multiple pregnancies: on average, 1.3 and 1.8 embryos were replaced in the PGD-A and non-PGD-A groups, respectively. Had they transferred a single embryo in both arms of the study, they would have prevented multiple pregnancy and perhaps even seen a more dramatic difference between the groups.

In conclusion, this multicenter, prospective RCT in patients of advanced maternal age demonstrates that PGD-A significantly improves the chances of a live birth after the first treatment attempt, while dramatically reducing miscarriage rates. There is no benefit of PGD-A for cumulative delivery rates, as might be expected, but neither a detrimental effect. For those patients without any euploid embryos (31%), application of PGD-A may avoid a high risk of miscarriage (39%) and unnecessary extra transfer cycles, whereas for those with euploid embryos, it reduces the time to a successful pregnancy with minimal risk of loss.

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http://dx.doi.org/10.1016/j.fertnstert.2017.03.015

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