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The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion

Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

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The value of preimplantation genetic testing for aneuploidy (PGT-A) as a screening test for in vitro fertilization (IVF) patients has yet to be determined. Several studies demonstrate higher birth rates after aneuploidy testing and elective single-embryo transfer (eSET), suggesting the potential for this testing to decrease the risk of multiple gestations, though these studies have important limitations. (Fertil Steril® 2018;109:429–36. ©2018 by American Society for Reproductive Medicine.)
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INTRODUCTION

Traditionally, morphology-based grading had been the primary technique used in in vitro fertilization (IVF) to evaluate and select the most competent embryos for transfer. Technologies have been developed in the fields of genomics, transcriptomics, proteomics, metabolomics, and time-lapse imaging to try to assist in the selection of the best embryos. However, a focus has been on analysis of 24-chromosome copy number for evaluation and transfer of only diagnosed euploid embryos, also known as preimplantation genetic testing for aneuploidy (PGT-A). Several molecular techniques have been utilized during IVF cycles to determine ploidy including fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), array CGH (aCGH), digital polymerase chain reaction (dPCR), single-nucleotide polymorphism (SNP) array, real-time quantitative PCR (qPCR), and next-generation sequencing (NGS). These technologies vary in terms of cost and time to completion, and few of these methods allow for fresh embryo transfer.

The earliest iterations of PGT-A evaluated a subset of the chromosomes primarily using FISH to examine 5-10 unique chromosomes. Despite the hypothesis that transfer of only euploid embryos should improve IVF outcomes, all but one randomized controlled trial (RCT) of this initial approach failed to demonstrate a benefit (1, 2). Since 24-chromosome techniques have become available, there have been few well-designed studies providing Level-I evidence regarding IVF pregnancy outcomes in select populations with these techniques. Several opinion pieces have discussed advantages and disadvantages of PGT-A (3, 4). The aim of this communication is to review the current evidence and to provide guidance for the use of PGT-A in IVF.

METHODS

Studies were eligible if they met one of the following criteria: primary evidence (clinical trials) that assessed the effectiveness of a procedure correlated with an outcome measure (pregnancy,

ovulation, or live-birth rates); meta-analyses; and relevant articles from bibliographies of identified articles. Final inclusion or exclusion decisions were made on examination of the articles in full. Disagreements about inclusion among reviewers were discussed and resolved by consensus.

A combination of the following medical subject headings or text words were used: preimplantation genetic screening; PGS; preimplantation genetic testing; PGT; preimplantation genetic diagnosis; PGD; genetic screening; aneuploidy; genetic testing/ methods; comprehensive chromosome screening; comprehensive chromosome analysis; next-generation sequencing; next-generation screening; NGS; comparative genomic hybridization; CGH; array comparative genomic hybridization; aCGH; single nucleotide polymorphism; SNP; polymerase chain reaction; PCR; quantitative polymerase chain reaction; quantitative real-time polymerase chain reaction; real-time quantitative polymerase chain reaction; qPCR; dPCR; whole genome sequencing; oligonucleotide array sequence analysis; microarray analysis; trophectoderm; blastocyst; embryo biopsy; embryo transfer; embryo selection; in vitro fertilization; in vitro fertilization; IVF.

RESULTS
Clinical Outcomes in Favorable-prognosis Patients

The literature search revealed only three RCTs with relatively small sample sizes, several retrospective cohort studies, and meta-analyses. A 2012 pilot study randomized 112 favorable-prognosis patients (age <35 years, tubal or male factor infertility, and no prior IVF treatment) to either day-5 aCGH after trophectoderm biopsy plus morphology assessment or traditional morphology assessment alone for selection of the single best embryo on day 6 (5). Ongoing pregnancy rates were significantly higher in the aCGH group compared with the traditional morphology group (69.1% vs 41.7%, P=.009). Of note, time to pregnancy was not reported, nor was total reproductive potential of the cycle. There was no statistically significant difference in miscarriages or multiples between the groups, though the study was not powered to address these outcomes. Biopsy for aCGH could not be completed in 32 blastocysts in the study group due to embryo degeneration or poor morphology, and failure of amplification resulting in "no signal" after biopsy occurred in eight blastocysts. Interestingly, for these favorable-prognosis patients, the authors found a blastocyst aneuploidy rate of 44.9% (191/425 biopsied blastocysts). The authors acknowledged their small numbers and limited study population, but concluded that outcomes with elective single-embryo transfer (eSET) are substantially improved with the addition of aCGH testing to traditional screening methodology.

Another group of investigators performed the other two RCTs, comparing pregnancy rates after transfer of morphologically graded embryos (controls) vs euploid embryos, based on comprehensive chromosome screening (CCS) (6). First, they hypothesized that SET with a euploid embryo would result in an equivalent pregnancy rate compared with double-embryo transfer (DET) of morphologically graded embryos. There were 175 patients (mean age 35.1 and 34.5 years for the study and control groups, respectively) who were eligible for randomization based on having at least two expanded blastocysts (most were randomized on day 5, but some did not have adequate blastocysts until day 6 of embryo development). The overall rate of aneuploidy was 31% (162/521) in the study group (mean maternal age = 35.1 ± 3.9 years). The primary outcome of ongoing pregnancy beyond 20 weeks was similar between the study and control groups (60.7% [54/89] vs. 65.1% [56/86]). The secondary outcome of clinical miscarriage was also similar between the study and control groups, though the study was not powered to address this outcome. The multiple pregnancy rate for patients in the study group was significantly lower than in the control group (0% [0/54] vs. 53.4% [31/56]). The authors concluded that transfer of a single euploid blastocyst was non-inferior in terms of ongoing pregnancy rates compared with transfer of two blastocysts with an unknown chromosome status.

A second study by the same group randomized women with two or more blastocysts on day 5 to biopsy with CCS on day 5 and transfer on day 6 (n=72) or the control group with morphologic grading and embryo transfer on day 5 (n=83) (7). There was no significant difference in the mean maternal age or number of high-quality blastocysts between subjects and controls (7.1 and 6.2 blastocysts for the study and control groups, respectively). Patients in the control group had significantly more embryos transferred than the CCS group (2.0 vs 1.86, P<.001), which the authors explain was due to 10 patients in the study group having only one euploid embryo for transfer while all patients in the control group underwent DET. They report that clinical implantation rates were significantly higher in the CCS vs control group (79.8% [107/134] vs 63.2% [103/163], P=.002). The proportion of CCS screened embryos that progressed to delivery was also significantly higher than the control group embryos (66.4% [89/134] vs 47.9% [78/163], P=.001). Analysis of secondary outcomes demonstrated a higher delivery rate per cycle in the CCS vs control group (84.7% [61/72] vs 67.5% [56/83], P=.01). Based on the reported data, the calculated spontaneous abortion rates for the CCS and control groups were 8.9% and 21.1%, respectively, and twin rates were approximately 59.7% and 45.1%. The authors concluded that trophectoderm biopsy with rapid qPCR-based CCS improves the chance of sustained implantation and delivery rates over traditional embryo selection.

It is worth noting that there are significant limitations to these RCTs. Specifically, randomization occurred only for patients who had a number of good-quality blastocyst embryos, which likely means that these are favorable-prognosis patients. If randomization occurred at cycle start, some percentage of those in the PGT-A group would not have had embryos to biopsy or transfer, thus likely altering success rates in that cohort, based on intent-to-treat analysis. Also, two of these studies were performed at a high-volume PGT-A clinic, which may limit generalizability to smaller programs. Another limitation is that these studies may not be reflective of current clinical practice, as most clinics biopsy embryos on day 5 and 6, vitrify, and thaw in a later cycle. While vitrified-thawed cycles have been postulated to have some benefits, there are likely to be some embryos that do not survive the thaw. In addition, instead of qPCR or aCGH that was used in these RCTs, many clinics now use NGS technology due to potential increased efficiency and precision and decreased cost (8). Thus, these studies may have limited current applicability due to out-of-date technological and practice changes. Results were not stratified by age (underpowered).

Analysis of data from national assisted reproductive technology (ART) surveillance systems from 2011-2012 has found that the use of PGT is not associated with improved rates of clinical pregnancy or live birth after fresh autologous blastocyst transfer among women aged ≤37 years, irrespective of the indication (9, 10). Most of these PGT-A cases from this period likely used FISH technology, which is rarely used today. However, PGT-A of embryos appeared to improve the likelihood of having a live birth among women >37 years, with 21 cycles (or 35 embryo transfers) as the number needed to treat (NNT) with PGT-A to have one additional live birth (10). Cycles that were intending PGT-A were more likely to reach embryo transfer in all age groups, but more significantly in women aged >37. This likely indicates that these women are patient cohort with a better prognosis,

and makes it difficult to isolate the benefit of PGT-A vs the intrinsic likelihood for success in these patients. A retrospective study from a large US clinic from 2010-14 found similar results with autologous fresh non-PGT-A cycles vs frozen cycles with PGT-A tested euploid embryos (11). When looking at clinical pregnancy, miscarriage, or live-birth rates, there was no difference between PGT-A and non-PGT-A cycles for women aged ≤ 37 years, and for women aged >37 years, there was no difference when comparing on a per-cycle basis.

Other Subsets of Patients

Age. There was one RCT that focused on women with advanced maternal age (38-41 years old), randomizing prior to cycle start to routine blastocyst transfer versus a PGT-A group that had a biopsy of a single blastomere on day 3 with transfer on day 5 (12). The live-birth rate was significantly higher in the PGT-A group when analyzed per transfer (52.9% vs 24.2%, P=.0002) and per cycle (36% vs 21.9%, P=.031). Of note, only 68% of the PGT-A patients had a transfer vs 95% in the control group (P=.001). The miscarriage rate was significantly lower in the PGT-A group (2.7% vs 39%, P=.0007). Of all cleavage embryos that were biopsied, they had a result for 97.2%, and 78.6% of embryos were aneuploid. There was no statistical difference in live-birth rate when they included outcomes for FET cycles for the 6 months after the study (37% vs 33.3% in controls) and the time to pregnancy was 4.5 weeks with PGT-A and 5.8 weeks with controls (P = NS). Time to pregnancy resulting in live birth was estimated at 7.7 weeks for PGT-A group vs 14.9 weeks for controls.

Retrospective studies suggest a benefit of PGT-A testing, particularly in women up to age 43 years (improved live-birth rate per cycle start seen in women aged 38-40 years with PGT-A [13]) and implantation rates in women 40-43 years of age (implantation rate was 50.9% in euploid embryos compared with unscreened fresh [23.8%] and FET [25.4%] cycles) (14). The retrospective nature, inclusion criteria, and small numbers limit these studies; in particular, one study stratified groups by age, thus comparing only eight cycles per group in the oldest age cohort (10), while another only included women with euploid embryos to transfer (only 76 of 145 patients had euploid blastocysts to transfer [52.4%]) (11). Furthermore, there is potential bias because only good-prognosis patients who were able to have a biopsy would have been included in the PGT-A group. The authors in both groups believe the improved pregnancy success demonstrates a benefit of PGT-A; however, the study methodologies leave questions regarding these conclusions.

Regarding donor-oocyte IVF cycles, the benefit of PGT-A was considered in a cohort study of 31 PGT-A cycles compared with 39 control cycles. PGT-A cycles showed no statistical difference in ongoing/live-birth rates (64.4% vs. 54%) or in miscarriage rates (19.2% vs. 9.5%) (15). The small numbers likely explain the heterogeneity of the study, thus limiting statistical power. Another group demonstrated a 15% aneuploidy rate in PGT-A tested embryos from donor-oocyte cycles; yet clinical pregnancy rates decreased when PGT-A tested embryos were used (16). Thus, the role of

PGT-A for donor-oocyte cycles is unknown. There may be a role for PGT-A in donor-oocyte cycles when paternal age is >50 years, based on studies showing an increased risk of aneuploid embryos with increasing paternal age (16).

eSET. A clinical scenario in which PGT-A may be of significant benefit is to increase utilization of eSET. Identifying appropriate candidates for eSET without compromising pregnancy success is an active area of study. Many advocate PGT-A to increase the utilization of eSET in patients undergoing IVF (17). A 2015 study compared IVF success before and after a change in clinic protocol designed to decrease the number of embryos transferred in patients older than 35 years. eSET was offered in patients with fewer than two implantation failures if favorable embryo morphology and/or PGT-A screening occurred. There were no significant differences in clinical pregnancy rates per transfer pre- and post-change in protocol, but there was a significant increase in live-birth rates per embryo transfer cycle for the eSET/PGT-A recipients. However, only 43.6% of PGT-A cycles had at least one euploid embryo to transfer. When comparing live-birth rates per cycle, there was no significant difference between groups (20.9% without PGT-A vs. 24.4% with PGT-A).

Recurrent Pregnancy Loss

The mechanism of first-trimester pregnancy loss is largely due to aneuploidy, providing biologic plausibility for PGT-A. An analysis of a retrospective cohort study (118 PGT-A vs 188 expectant management) demonstrated similar clinical pregnancy rates and miscarriage rates between the two groups (18), though time to successful pregnancy was statistically shorter in the expectant-management group (3.0 vs 6.5 months, respectively). Of the PGT-A cohort, 77% were able to create embryos that were tested and, of those, 74% had at least one euploid embryo to transfer. This study is limited by its retrospective design, which makes it difficult to interpret potentially different clinical prognoses for those who did or did not pursue PGT-A.

A prospective study explored the relationship between ovarian reserve in recurrent pregnancy loss (RPL) patients and found that in women younger than 38 years, decreased ovarian reserve (defined as a cycle day-3 follicle-stimulating hormone level >10 IU/mL and/or antimüllerian hormone <1 ng/mL) resulted in a significantly lower likelihood of having a euploid embryo to transfer compared with women with normal ovarian reserve testing (19). These studies can assist in personalizing the counseling for patients considering PGT-A, regarding one's likelihood of successfully obtaining a euploid embryo from the technology. It is worth noting that the increased rate of aneuploidy with decreased ovarian reserve is likely not unique to the RPL population (20). However, to date, the literature has not suggested an improved live-birth rate using PGT-A in RPL patients.

Frozen-embryo Transfer Cycles

Due to logistical and cost requirements, the majority of clinics performing PGT-A currently do not process cells for ploidy assessment in-house. As blastocysts can be biopsied on day

5, 6, or 7, most euploid blastocysts are transferred in cryopreservation (vs fresh) cycles. Data from one retrospective cohort study support equal or superior reproductive potential for frozen euploid blastocyst transfers (vs fresh euploid blastocyst transfers) with higher implantation and live-birth rates, and lower miscarriage rates (21). Additional plausible benefits may include a lower incidence of both ovarian hyperstimulation syndrome and multiple gestation if eSET is utilized. Limitations include the retrospective nature of the study, and potential limited generalizability due to the need for good-quality blastocysts for inclusion in this study.

Day-5 versus Day-6 Biopsy

When comparing outcomes for blastocysts biopsied on day 5 (n=730) vs day 6 (n=441), the aneuploidy rate was not significantly different in the day-6 group (69.9% versus 61.9%) (22). The age of the women in the two groups was not significantly different (mean age 38.5 years). Embryos biopsied on day 5 could be transferred fresh on day 6 or frozen, but all day-6 embryos were frozen for future FET. The implantation rate, clinical pregnancy rate, and live-birth rates were not significantly different. This study suggests that the developmental rate of euploid blastocysts that form on day 6 may be approximately as likely to result in live birth as those that form on day 5, although day-6 blastocysts may require cryopreservation for later transfer in an FET cycle.

PGT-A with Preimplantation Genetic Testing for Monogenetic Disorders (PGT-M)

Preimplantation genetic testing for monogenic disorders (PGT-M) predates PGT-A for embryo aneuploidy. With improvements in deoxyribonucleic acid (DNA) amplification techniques, it became possible to perform simultaneous PGT-M/PGT-A. One study compared outcomes of PGT-M/PGT-A vs PGT-M alone, and found that ~50% of PGT-M-unaffected embryos were aneuploid (mean maternal age 32.4 years) (23). Accordingly, the authors reported implantation rate of 75% vs 53% (P=.19) and live-birth rates of (59.4% vs 37.5%) in the PGT-M/PGT-A group, with miscarriage rates of 20% vs 40% (P=.56). Patients undergoing PGT-M/PGT-A ultimately will have fewer embryos remaining for transfer after testing, but potentially will have a better assessment and higher reproductive potential with those remaining embryos, though further studies are needed in this population.

Thaw/Warm, Biopsy, and Recryopreserve for PGT-A

Patients with previously cryopreserved unbiopsied embryos may wish to thaw/warm their embryos for biopsy and testing followed by use or repeat cryopreservation. Reasons for this include previous miscarriage, disease discovery, family balancing, or desire to utilize new technology. While fresh biopsy is preferable, reproductive outcomes did not seem significantly compromised with respect to implantation rate, clinical pregnancy rate, or biochemical loss in one study on surviving euploid embryos after a sequence of warm/thaw, biopsy, (re)vitrification, and (re)warming (24). There was no comparison of live-birth rates in this group. One study found

that the survival rate was lower for the second warming (87.5% vs 98.3% in first thaw/warming, P=.035), but some of the embryos had been slow frozen on the first freeze. In contrast to embryos that were warmed for an initial biopsy, embryos warmed for a second biopsy (i.e., after initial "no read," n= 3) did not perform well; in fact, none implanted in this study. Another study with a small sample size (underpowered) reported that for blastocysts that were warmed, biopsied, and transferred within 2 days (day 6 or day 7 of progesterone), ongoing pregnancy rates were 35.3% for age ≤35 (n=17), 40% for age 36-44 (n=16), and 100% for donor egg (n=2) (25). Many patients may benefit from warming embryos for preimplantation screening, though, again, they may expect a reduction in the number of embryos available for transfer.

Male Factor Infertility

One study compared rates of blastocyst aneuploidy for men with normal semen analyses (concentration >19 M/mL, motility >30%, morphology >30%) to men with oligozoospermia (concentration <6 M/mL) and reported a 3-fold increase in sex chromosome abnormalities in the oligozoospermia group, regardless of oocyte age (26). The authors acknowledged that intracytoplasmic sperm injection (ICSI), which is traditionally used for PGT-A/PGT-M cycles, could increase aneuploidy by affecting sperm nuclear decondensation or by destabilizing the oocyte spindle apparatus, but reported no difference in blastocyst aneuploidy rates for men with normal semen analyses who underwent IVF/PGT-A using conventional vs ICSI fertilization. In oligozoospermic men, ICSI did not increase overall aneuploidy (vs conventional) but did increase aneuploidy in chromosomes 1, 2, 11, and 18. The authors conclude that patients with severe oligozoospermia have a higher rate of sex chromosome aneuploidy and may choose to pursue PGT-A; more investigation is warranted for other parameters, such as morphology.

Ethnicity

Although IVF outcomes have been reported to vary by ethnicity, a 2016 study found no difference in aneuploidy rates based on maternal ethnicity as defined by ancestry informative markers (AIMs) (27). Limitations include the lack of paternal AIMs data, the current AIMs' inability to identify ethnicity subgroups, and that the majority of the study population was of European descent. A wider group is needed for future study, but aneuploidy risk stratification by ethnicity is not currently indicated.

Neonatal and Childhood Outcomes

Obstetric, neonatal, and early childhood outcome data seem reassuring thus far, though much has focused on PGT-M (single gene) rather than PGT-A (aneuploidy). The PGT-M vs PGT-A parental groups are often inherently different in that most patients undergoing PGT-M do not have concomitant infertility. Nonetheless, kindergarten-aged PGT-M offspring perform as well as their IVF/ICSI and naturally conceived

peers on measures of cognition (Wechsler Preschool and Primary Scale of Intelligence™), motor skills (Movement ABC) and psychosocial development (Child Behavior Checklist [CBCL] and Caregiver-Teacher Report Form [C/TRF]) (28, 29).

A cohort study from Denmark noted that adverse obstetric and neonatal outcomes seemed more related to the parental condition than the technology used to treat the condition, though PGT-M pregnancies had more placenta previa than spontaneously conceived pregnancies. PGT-M pregnancies with monogenic disorders demonstrated more low birth weight, preterm prelabor rupture of membranes, placenta previa, cesarean delivery, and neonatal intensive care unit stays than both IVF/ICSI and spontaneously conceived pregnancies; however, the PGT-M offspring did not differ in these variables when compared with their unaffected siblings who were not from PGT-M cycles, suggesting an underlying familial/parental risk milieu (30).

Cost-effectiveness

Cost-effectiveness for PGT-A is difficult to quantify, as cycle costs and insurance coverage vary considerably. It is difficult to quantify the intangible costs of miscarriage and failed implantation, and many studies do not consider all obstetric, neonatal, and ongoing costs of disease/aneuploidy. One study found that applying PGT-A to patients with unexplained RPL (n=232) was not cost-effective when compared with expectant management (n=302); though PGT-A decreased miscarriage rates (7% vs 24%), the live-birth rate was not improved (40% vs 55%) (31). More research is needed, and clinicians should tailor their recommendations to the preference and situation of the individual patient.

Concerns with Test Characteristics

Mosaicism. Mosaicism refers to two or more cell populations with different chromosomal complements being present within the same embryo. Mosaicism was first identified as a common phenomenon in cleavage-stage embryos, although the exact prevalence of mosaicism in embryos is unknown. Embryonic mosaicism is believed to be a confounder when trying to interpret PGT-A results, as mosaic embryos are currently classified as either aneuploid mosaic or diploid-aneuploid mosaic, the latter of which is influenced more by early embryo-cleavage events when chromosomal segregation occurs (32). Trophectoderm biopsy, whereby 4-10 cells are removed from the embryo for chromosomal analysis, has provided several advantages over cleavage-stage biopsy, including the purported improved detection of mosaicism. Numerous studies have demonstrated the utility of aCGH for use in PGT-A (33, 34); however, the ability of aCGH to detect mosaicism is dependent on the percentage of aneuploid cells in the trophectoderm biopsy. Analytic platforms for PGT-A, such as NGS, now have the ability to detect mosaicism molecularly. The higher rates of reporting mosaicism by NGS compared with aCGH have called into question the validity of a diagnosis of mosaicism. Mosaic embryos could implant and generate euploid offspring; however, they may implant at a lower success rate (35, 36). An Italian

series reported 18 cases of transfer of mosaic embryos resulting in the birth of eight healthy infants (35). Although NGS has the advantage of high accuracy with increased throughput and decreased cost compared with aCGH, more research is needed to elucidate the mosaicism phenomenon further (37, 38). Additional insight into the self-correction of aneuploidy and mosaic embryos, either by post-zygotic chromosome loss, chromosome gain, mitotic nondisjunction, or trisomic rescue, requires further investigation (39–41).

There is a recent, prospective, double-blind study comparing the reliability of qPCR and aCGH, two widely used PGT-A methods, for blastocyst-stage aneuploidy screening (42). The study demonstrated that qPCR and aCGH had similar sensitivities (98.2% vs 98.8%, respectively), but found that qPCR had slightly higher specificity compared with aCGH (99.9% vs 99.6%, P=.01). NGS and SNP microarray are also commonly used. SNP microarray has the added benefit of indicating if the source of aneuploidy is from the sperm or egg, reliably detecting triploidy and tetraploidy, and detecting low levels of mosaicism. Due to differences in laboratory protocols and quality controls, current data do not exist to conclusively determine the superiority of any platform.

Embryo damage. There are few data on embryo biopsy techniques used in PGT-A; however, it is generally accepted that trophectoderm biopsy has less impact on embryo viability than cleavage-stage biopsy (43). This is because even though more cells are removed during trophectoderm biopsy, it represents a smaller percentage of embryo mass and, by definition, trophectoderm biopsy removes only trophectoderm cells and not cells that have any fetal fate. Conversely, cleavage-stage biopsy occurs at a time when cell lineage has not yet been established and the cell removed could potentially impact viability of the embryo and the fate of the fetus. Available data evaluating the impact of cleavage-stage embryo biopsy show a significant developmental insult that is associated with the biopsy process itself, thereby inflicting trauma to the developing embryo and relative reduction in embryo implantation and progression to delivery (44). There was potential selection bias in this study, given that only poorly developing embryos biopsied on day 3, whereas normally developing embryos were allowed to grow until day 5 or 6 before biopsy. The impact of biopsy of the trophectoderm is not well understood and, given the importance of the trophectoderm for implantation, damage to the trophectoderm may impact this critical event.

Gaps in Knowledge

Other potential advantages and disadvantages exist with PGT-A, though there are limited data to support or refute these. For example, PGT-A testing may lower the risk of aneuploidy detected during pregnancy or after birth. Another consideration is that identification and discard of aneuploid embryos could potentially lessen the burden of excess embryos cryopreserved. Also, identifying euploid embryos may decrease the time to pregnancy by focusing embryo-transfer cycles only using euploid embryos to select populations; this may be helpful in older women, those who want big

families, or cancer patients. On the other hand, time to pregnancy may be faster in patients who conceive after a fresh transfer without PGT-A, as only those who did not conceive would pursue subsequent FETs with tested euploid embryos. Lastly, while controversial, sex selection is a potential benefit. Ideally, more RCTs that randomize patients at cycle start and evaluate cumulative live-birth rates are needed to elucidate some of these answers.

There are potential disadvantages to using PGT-A, such as the need for increased resources and up to 8 cumulative hours of labor for the embryology team for each biopsy case (45). Further, not all embryos will survive in culture to the blastocyst stage for biopsy, though hypothetically they may have resulted in a healthy live birth if they had been transferred in the cleavage or early blastocyst stage. Given the uncertainty about self-correction, false positive PGT-A results, and/or accuracy of a mosaic diagnosis, there is concern that one may be discarding embryos that may have resulted in healthy babies (35). More data are needed about cumulative pregnancy rates from one retrieval cycle, effects of PGT-A on miscarriage rates, and defining which patient groups could benefit from this technology.

CONCLUSIONS

The value of PGT-A as a universal screening test for all IVF patients has yet to be determined. Some studies reported here provide important perspectives on the value of 24-chromosome testing, demonstrating higher birth rates after aneuploidy testing and eSET in the primary embryo transfer of favorable-prognosis patients, suggesting the potential for this testing to increase eSET utilization and further decrease the incidence of multiple gestations. However, these studies have important limitations and there remain questions about appropriate patient selections and testing platforms.

- Patients participating in the RCT are generally favorably responding subjects who have produced blastocysts for biopsy and analysis. A broader selection of patients with randomization at cycle start rather than blastulation would more appropriately address the applicability of wider use of this technology. The randomized trials were performed in centers with broad and deep experience in embryo biopsy and specimen preparation. The ability to expand these techniques to centers with less experience has yet to be established.
- One RCT in patients aged 38-41 demonstrated improved live-birth rates per cycle with a day-3 single-blastomere biopsy and PGT-A.
- The extremely challenging questions of false-positive testing, embryonic damage, and loss of euploid embryos between day 3 and blastulation remain unanswered.
- To date, there are very few studies directly comparing the specific laboratory techniques for assessing ploidy. Future studies are warranted to determine if any platforms are superior.
- Other important considerations about PGT-A that must be addressed by further research include: cost-effectiveness;

the role and effect of cryopreservation, time to pregnancy, utility in specific subgroups (such as recurrent loss, prior implantation failure, advanced maternal age, etc.); cumulative success rates over time; and total reproductive potential per intervention. Unfortunately, at the time of this publication, there are currently very few randomized trials registered to elucidate these answers.

Large, prospective, well-controlled studies evaluating the combination of multiple approaches (genomics, time-lapse imaging, transcriptomics, proteomics, metabolomics, etc.) for enhanced embryo selection applicable in a more inclusive IVF population are needed to determine not only the effectiveness, but also the safety and potential risks of these technologies. PGT-A will likely be part of a future multidimensional approach to embryo screening and selection. At present, however, there is insufficient evidence to recommend the routine use of blastocyst biopsy with aneuploidy testing in all infertile patients.

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