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REVIEW

Preimplantation genetic testing for aneuploidy: A Canadian Fertility and Andrology Society Guideline

BIOGRAPHY

Crystal Chan is a Reproductive Endocrinology and Infertility Specialist practising at Mount Sinai Fertility, Toronto, Canada. She is a clinician investigator and Assistant Professor in the University of Toronto Department of Obstetrics and Gynaecology. She serves as the clinical lead of the Mount Sinai Fertility Preimplantation Genetic Testing programme.

Crystal Chan[1,2,*], Michelle Ryu[1], Rhonda Zwingerman[2]

KEY MESSAGE

Existing evidence on PGT-A was reviewed and used to generate evidence-based practice recommendations. In patients able to generate two or more blastocysts, PGT-A was found to increase implantation rate and ongoing pregnancy or delivery rate per transfer. Limitations of the technology were reviewed, and important areas for future research identified.

ABSTRACT

The objective of this guideline from the Canadian Fertility and Andrology Society is to synthesize the evidence on preimplantation genetic testing for aneuploidies (PGT-A) using trophectoderm biopsy and 24-chromosome analysis and to provide clinical recommendations using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. To date, randomized controlled trials have been limited to good-prognosis patients who were able to generate two or more blastocysts for biopsy. In this specific population the GRADE analysis of PGT-A shows an increase in the implantation rate and ongoing pregnancy or delivery rate per transfer. Clearly, it is difficult to generalize from this subgroup of patients to the infertility population at large. As a result, the application of PGT-A should be individualized, and patient factors such as age and ability to generate embryos will influence decision-making. Comprehensive patient counselling and informed consent are imperative before undertaking PGT-A. Potential benefits must be weighed against the costs and limitations of the technology, including the risk of embryo damage, false positives, false negatives and the detection of embryonic mosaicism. Future research is required, especially with regard to the use of PGT-A in poorer prognosis patients, and with respect to reporting outcomes per cycle start and cumulatively per retrieval.

[1] Mount Sinai Hospital, Sinai Health System, Toronto, Canada
[2] Department of Obstetrics & Gynaecology, University of Toronto, Toronto, Canada

*Corresponding author. E-mail addresses: cryssie.chan@mail.utoronto.ca, Crystal.Chan@sinaihealth.ca (C. Chan). https://doi.org/10.1016/j.rbmo.2020.10.020 1472-6483/
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KEY WORDS

Chromosome aberrations
Infertility
IVF
Mosaicism
Recurrent miscarriage
Spontaneous abortion

INTRODUCTION

Preimplantation genetic testing (PGT) analyses the DNA of an embryo for genetic abnormalities prior to implantation. Results of the analysis are then used to decide whether or not to transfer the embryo. This technology has three main applications: PGT for aneuploidy (PGT-A) (previously preimplantation genetic screening, PGS), PGT for monogenic or single-gene defects (PGT-M) (previously preimplantation genetic diagnosis, PGD), and PGT for chromosomal structural rearrangements (PGT-SR). The latter two applications are used for patients who are known or suspected carriers of single-gene defects or structural rearrangements, respectively. PGT-A is offered to patients who are not necessarily carriers of any genetic condition, to screen their embryos for numerical chromosomal abnormalities before embryo transfer. The purpose of this guideline is to provide clinical recommendations on PGT-A based on the best available evidence.

METHODS

This guideline was developed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (*Guyatt et al., 2011*). A comprehensive literature search strategy was developed with the assistance of an information specialist. The following electronic bibliographic databases were searched up to December 2019: (i) Ovid MEDLINE, (ii) Ovid EMBASE, (iii) EBM Reviews – Cochrane Central Register of Controlled Trials, (iv) EBM Reviews – Cochrane Database of Systematic Reviews, and (v) PubMed (Non-MEDLINE records only). The search strategy was structured based on the *Peer Reviewed Electronic Search Strategies (PRESS) 2015 Guidelines* (*McGowan et al., 2016*). No restrictions were applied to language of publication, age or publication year. A combination of the following Medical Subject Headings (MeSH), Emtree terms and keywords were used: preimplantation diagnosis; PGS; PGT-A; in vitro fertilization; IVF; infertility; sterility; chromosome aberrations; aneuploid; abortion; spontaneous abortion; habitual abortion; embryo loss; fetal death; recurrent miscarriage; and mosaicism. All references and duplicate records were managed using EndNote X8 citation (www.endnote.com) management software. Conference abstracts were excluded from review. Full articles were screened for inclusion or exclusion by two independent reviewers. Additional articles were found through hand searching and reviewing the references of relevant papers.

HISTORICAL CONTEXT

The original application of PGT was to provide carriers of hereditary diseases with an alternative to prenatal testing and pregnancy termination by genetically screening and selecting embryos prior to transfer to prevent disease transmission. The very first PGT case was performed for medically indicated sex selection, specifically to select female embryos to transfer from carriers of X-linked recessive disorders (*Handyside et al., 1990*). PGT-M was further developed to test embryos for specific single-gene disorders (*Handyside et al., 1992*). The next application of the technology was PGT-A, to screen the chromosomal complement of an embryo to minimize the chance of aneuploid embryos being transferred. The proposed benefits of screening preimplantation embryos for aneuploidy were to improve embryo selection over assessment of standard morphological parameters so as to enhance the likelihood that a transferred embryo would lead to a healthy live birth (*Munné et al., 1993*).

The first PGT-A was performed using fluorescence in-situ hybridization (FISH), which enabled visual examination of blastomeres for chromosomal complement using multiple DNA probes labelled with different fluorochromes (*Munné et al., 1993*). However, because of intrinsic limitations of the platform with respect to the number of fluorochromes, only a few chromosomes could be tested on a given sample at a time. Therefore, this technology was only useful for identification of the selected aneuploidies (usually of chromosomes 13, 18, 21, X and Y).

Despite earlier optimism that FISH-based PGT-A could improve IVF outcomes, a multicentre randomized controlled trial (RCT) (*Mastenbroek et al., 2007*) and then a meta-analysis of nine RCT by Mastenbroek and colleagues showed a detrimental effect of PGT-A on IVF outcomes (*Mastenbroek et al., 2011*). PGT-A significantly reduced the live birth rate (LBR) for women of advanced age (risk difference –0.08; 95% confidence interval [CI] –0.13 to –0.03) and women with recurrent implantation failure (risk difference –0.18; 95% CI –0.33 to –0.03). Furthermore, there was no improvement in miscarriage or multiple pregnancy rates. Eight out of nine studies used cleavage-stage embryo biopsy, and only one used the newer technology of trophectoderm biopsy from blastocyst-stage embryos. The authors speculated on possible reasons for the apparent inefficacy of PGT-A, including possible harm from the invasive biopsy, inherent limitations of FISH, which only analyses a few chromosomes, and inaccurate results due to significant mosaicism of cleavage-stage embryos.

However, even as this meta-analysis was being published, the field was already abandoning FISH-based PGT-A because of its inherent limitations. The incomplete nature of FISH cytogenetic analysis was replaced by platforms that allowed for whole-genome amplification and full 24-chromosome analysis, also known as comprehensive chromosome screening (CCS). These platforms include array comparative genomic hybridization (aCGH), single-nucleotide polymorphism (SNP) array, quantitative polymerase chain reaction (qPCR) and, most recently, next-generation sequencing (NGS). A discussion of the differences among these methods with respect to their ability to detect polyploidy, uniparental disomy, balanced translocations and mitochondrial copy number is beyond the scope of this guideline; however, it should be noted that NGS is currently the most commonly used platform (*Kim et al., 2018*).

As FISH was being supplanted by newer CCS platforms, embryo biopsy techniques were also improving. With advances in laboratory techniques and extended culture, trophectoderm biopsy of blastocysts became preferred over blastomere biopsy of cleavage-stage embryos. The transition from slow freezing to vitrification enabled blastocysts to be reliably cryopreserved while awaiting PGT results without compromising implantation rates (*Harper and Sengupta, 2012*). There is biological plausibility for trophectoderm biopsy being safer than blastomere biopsy because a smaller proportion of cells are removed. A randomized study in women aged under 35 years in whom 116 pairs of sibling embryos were biopsied by a

single senior embryologist at either the blastocyst or cleavage stage followed by double-embryo transfer demonstrated that implantation rates decreased after blastomere biopsy but not after trophectoderm biopsy (R. T. *Scott et al., 2013*). Trophectoderm biopsy may also lead to a more accurate analysis because more DNA is available. A comparison study demonstrated that the positive predictive value of a euploid diagnosis to predict successful implantation is significantly higher with trophectoderm biopsy than with blastomere biopsy (48% versus 29%, $P = 0.0016$) (*Scott et al., 2012*).

Given that the current standard of care for PGT-A is trophectoderm biopsy with CCS, the scope of this document will be to review the evidence using these techniques. Cleavage-stage biopsy, cytogenetic platforms that do not screen for all 24 chromosomes and emerging non-invasive methods will not be reviewed in this document.

POTENTIAL INDICATIONS FOR PGT-A

All patients, irrespective of oocyte age or underlying diagnosis, have a risk of generating aneuploid embryos (*Franasiak et al., 2014*). Therefore, suggested indications for PGT-A have encompassed patients in a variety of clinical scenarios. Commonly proposed indications include prior aneuploid conception, recurrent pregnancy loss (RPL), recurrent implantation failure, unexplained infertility and advanced reproductive age (ARA). Other suggested indications include male factor infertility, reduction of time to pregnancy, and to aid in embryo selection in patients with multiple blastocysts (*Sermon et al., 2016*). PGT-A may also be used to increase the uptake of elective single-embryo transfer (eSET) and therefore decrease the incidence of multiple births and its resultant complications (*Forman et al., 2013*). PGT-A may be considered in cases using gestational carriers to promote eSET and minimize the risk of multiple birth in this third-party population. PGT-A can also be performed alongside PGT-M to screen unaffected or carrier embryos for ploidy prior to transfer (*Sermon et al., 2016*). Lastly, some proponents support universal PGT-A for all patients undergoing IVF, including egg donor cycles (*Sermon et al., 2016*).

ASSESSING THE IMPACT OF PGT-A AND THE IMPORTANCE OF OUTCOME MEASURES

The degree of benefit or harm from PGT-A depends on the particular population in which PGT-A is applied and the values prioritized by individual patients. While PGT-A might be associated with benefits such as fewer failed embryo transfers, shortened time to live birth and fewer pregnancy losses, it could also result in negative outcomes such as a reduction in cumulative births from damage to or loss of blastocysts from the trophectoderm biopsy, or false positives leading to the non-use of viable embryos.

The proposed benefits of PGT-A are to assist with embryo selection and enhance the likelihood that a transferred embryo will lead to a healthy live birth. Given the emphasis on embryo selection, existing PGT-A RCT using CCS are limited to patients randomized at the blastocyst stage, with more than one blastocyst available for transfer (*Forman et al., 2013*; *Munné et al., 2019*; R. J. *Scott et al., 2013*). Accordingly, outcomes, when reported per embryo transfer, reflect the largest potential benefit of PGT-A in less generalizable circumstances. It is uncertain if such benefit would be maintained if a study randomized at cycle start a wider range of patients some of whom were not able to generate multiple blastocysts.

In order to assess the potential for harm resulting from PGT-A, studies would need to report the cumulative LBR (CLBR) per cycle started, inclusive of all embryos that result from the cycle (*Maheshwari et al., 2015*). This outcome measure would account for potential limitations of the technology such as harm from the biopsy, misdiagnosis and embryo wastage. Two of the randomized trials discussed below (*Forman et al., 2014*; *Yang et al., 2013*) include up to one subsequent transfer if the first transfer failed to result in a live birth; however, no randomized studies to the current authors' knowledge have followed more than one subsequent transfer or continued with embryo transfers until a live birth was achieved or the entire embryo cohort was exhausted. There have been a number of cohort studies that have reported on the outcome of CLBR with variable follow-up periods. A retrospective cohort study by Murphy and colleagues that reported on the CLBR per cycle start and included all subsequent embryo transfers found no difference for women aged 38 years or older (37.8% versus 37.3%; P = non-significant [NS]) and a significantly lower CLBR in women less than 38 years old (49.4% versus 69.1%; $P < 0.001$) (*Murphy et al., 2019*). A 2015 cohort study by Ubaldi and co-workers reported cumulative delivery rates per oocyte retrieval (to a maximum of 12 months) with and without PGT-A and found no significant difference between groups (24.4% versus 20.9%; P = NS) (*Ubaldi et al., 2015*). The mean female age in that study was 39 years. More recently, Sacchi and colleagues published a prospective cohort study of women aged 38–44 years with and without PGT-A that included up to 2 years of follow-up (*Sacchi et al., 2019*). They found that the CLBR per oocyte retrieval was not significantly different between groups (26.3% versus 24.0%; P = NS).

To mitigate the risk of multiple births and their sequelae, eSET is generally recommended, particularly in a good-prognosis patient population. The practice of eSET should be considered the standard of care when PGT-A has been performed and the embryo being transferred is euploid. Some studies (*Forman et al., 2014*, *2013*; *Yang et al., 2013*) on PGT-A reported multiple birth rate as an outcome measure. One randomized study (*Forman et al., 2013*) compared single euploid embryo transfer to double unscreened embryo transfer, and found a predictable increase in multiple births in the double-embryo transfer arm. Although PGT-A itself does not lead to reduced multiple births, PGT-A promotes the use of eSET.

EVIDENCE FOR USE IN CLINICAL PRACTICE

PGT-A and infertility

There are five published RCT that have examined the impact of PGT-A with trophectoderm biopsy on the outcome of the first embryo transfer (*Forman et al., 2013*; *Munné et al., 2019*; *Ozgur et al., 2019*; R. J. *Scott et al., 2013*; *Yang et al., 2012*), two of which have published subsequent follow-up studies reporting on the cumulative outcomes up to one additional frozen embryo transfer (FET; *Forman et al., 2014*; *Yang et al., 2013*). All the studies enrolled good-prognosis patients with blastocysts available for biopsy and PGT-A.

Yang and colleagues randomized participants undergoing their first IVF cycle to PGT-A using aCGH and a single fresh euploid embryo transfer (n = 55) or a fresh blastocyst transfer based on morphological assessment alone (n = 48) (*Yang et al., 2012*). All female patients were less than 35 years old with no previous pregnancy losses, regular cycles, normal karyotypes and normal uterine contours. The mean number of blastocysts available was 8.3 in the PGT-A group and 8.1 in the morphology group. All patients had a fresh embryo transfer on day 6. The ongoing pregnancy rate (OPR) beyond 20 weeks' gestation was significantly higher in the PGT-A group (69.1% versus 41.7%). All patients in the above study had at least one embryo cryopreserved. In a follow-up study, the authors reported the outcomes of the first FET in those participants who did not conceive with their fresh embryo transfer (*Yang et al., 2013*). In the PGT-A arm, 15 patients had 22 euploid blastocysts transferred. In the morphology arm, 23 patients had 42 blastocysts transferred. The implantation rate was significantly higher in the PGT-A arm (65.0% versus 33.3%); however, the differences in OPR (66.7% versus 52.2%), twin rate (30.0% versus 16.7%) and early pregnancy loss rate (EPL) (0% versus 16.7%) did not reach statistical significance.

Forman and co-workers randomized participants undergoing their first or second IVF cycle to PGT-A using qPCR and a single euploid embryo transfer (n = 89) or a double-embryo transfer based on morphology alone (n = 86) (*Forman et al., 2013*). They included women under 43 years old with regular cycles, normal ovarian reserve, normal uterine contours and a body mass index of less than 30 kg/m^2 who had at least two expanded blastocysts suitable for transfer or cryopreservation by day 6. The mean number of blastocysts available was 5.8 in the PGT-A group and 5.3 in the morphology group. The average age of patients was 35.1 ± 3.9 years in the study group versus 34.5 ± 4.7 years in the control group (P = 0.5). In their intention-to-treat analysis, the implantation rate was not significantly different between the PGT-A and morphology groups (63.2% versus 51.7%). The OPR was also not significantly different (60.7% versus 65.1%). The EPL rate appeared to be lower with PGT-A but did not reach statistical significance (11.5% versus 20.0%). In the follow-up study, 17 participants had subsequent euploid FET (16 single-embryo and one double-embryo transfer) and 13 had subsequent unscreened FET (three single-embryo and 10 double-embryo transfers) (*Forman et al., 2014*). The CLBR up to one additional transfer was 69% in the PGT-A arm and 72% in the morphology arm; however, the rate of multiple births was significantly higher in the morphology/double-embryo transfer group.

Scott and co-workers randomized participants undergoing their first or second IVF cycle to PGT-A using qPCR and a fresh double euploid embryo transfer on day 6 (n = 72) or a fresh double embryo transfer based on morphology alone on day 5 of development (n = 83) (R. J. *Scott et al., 2013*). They included women under 43 years old with normal ovarian reserves and normal uterine contours who had at least two blastocysts on day 5. The average age of patients was equivalent in both groups (32.2 ± 0.5 years in the PGT-A group versus 32.4 ± 0.5 years in the control group). The mean number of blastocysts available was 8.0 in the PGT-A group and 7.9 in the morphology group. There was a difference in number of embryos transferred (1.8 ± 0.04 in the PGT-A group versus 2 ± 0.0 in the control group; $P < 0.001$), because some patients in the study group only had a single euploid embryo available for transfer. The implantation rate was higher in the PGT-A group (79.8% versus 63.2%; P = 0.002), as was the LBR per transfer (84.7% versus 67.5%; P = 0.01).

The Single Embryo TrAnsfeR of Euploid Embryo (STAR) trial was the largest, blinded, multicentered RCT comparing PGT-A using NGS with cryopreserved single euploid embryo transfer (n = 330) with cryopreserved single-embryo transfer based on morphology alone (n = 331) (*Munné et al., 2019*). They included women 25–40 years old with normal ovarian reserves, no more than two prior failed IVF cycles and/or one prior pregnancy loss, and at least two blastocysts suitable for biopsy and vitrification by day 6 of development. The mean number of blastocysts available was 7.4 in both the PGT-A and morphology groups. In the PGT-A arm, all good-quality blastocysts underwent biopsy for PGT-A and were vitrified, and the euploid embryo with the most favourable morphological assessment was transferred in a subsequent vitrified–warmed embryo transfer cycle. In the control arm, only the morphologically best blastocyst was cryopreserved without being biopsied and transferred in a subsequent cycle, while all remaining blastocysts in the cohort were biopsied for PGT-A before vitrification. The average age of the patients was similar in both arms (33.7 ± 3.6 years in the PGT-A group versus 33.8 ± 36 years in the control group). The OPR beyond 20 weeks were not significantly different between the PGT-A and control arms when analysed either per intention-to-treat (41.8% versus 43.5%) or per embryo transfer (50.0% versus 45.7%).

This study reported that miscarriage rates were not different between the two groups (9.9% versus 9.6%, P = 0.90); however, the authors unconventionally calculated the miscarriage rate using the total number of embryo transfers rather than the clinical pregnancy rate as the denominator. Re-analysing the data presented in the paper, the miscarriage rates (calculated as the number of clinical pregnancy losses divided by the number of clinical pregnancies) were still similar in both arms but higher than reported (16.4% [27/165] in the PGT-A arm and 17.1% [30/175] in the control arm). Similarly, pregnancy loss rates (calculated as the number of positive human chorionic gonadotrophin [HCG] results not resulting in an ongoing pregnancy divided by the number of pregnancies with a positive HCG result) were 29.4% (57/194) in the PGT-A arm and 28.9% (58/201) in the controls. This study was limited in design as they could not follow additional transfers or time to pregnancy, or compare CLBR between PGT-A and control cases because all blastocysts other than the morphologically best blastocyst in the control group underwent PGT-A before vitrification.

A sixth RCT of PGT-A was not included in this review because PGT-A was not used for embryo selection purposes as only the single best blastocyst (as per morphological scoring) underwent PGT-A in the treatment arm (*Ozgur et al., 2019*).

Summary statements:

1 RCT of PGT-A and trophectoderm biopsy have been limited to patient populations with at least two blastocysts available for biopsy/transfer.

2 To date there are no RCT of PGT-A with trophectoderm biopsy that have:

   (i) randomized patients at the start of the cycle
   (ii) been conducted in patient populations with only one blastocyst available for biopsy
   (iii) examined the CLBR, or
   (iv) examined the time to pregnancy/live birth.

Recommendations:

1 In patients with infertility undergoing IVF with at least two blastocysts, clinicians may consider the use of PGT-A to improve the ongoing pregnancy or delivery rate per embryo transfer.

Strength: weak.

Quality of evidence: moderate (TABLE 1).

1 In patients with infertility undergoing IVF with at least two blastocysts, clinicians may consider the use of PGT-A to reduce the risk of EPL per clinical pregnancy.

Strength: weak.

Quality of evidence: moderate (TABLE 1).

1 With euploid embryos available, eSET is recommended.

Strength: strong.

Quality of evidence: very low (expert opinion).

PGT-A and ARA

It is well established that aneuploidy rates increase with oocyte age. In a study of over 15,000 PGT-A-tested blastocysts, the mean aneuploidy rate in women less than age 35 was less than 40%, while in women over the age of 40, the aneuploidy rate exceeded 70% (*Franasiak et al., 2014*). To date no RCT has specifically evaluated the impact of PGT-A in women over the age of 40. However, to evaluate the use of PGT-A in women with ARA, the STAR trial performed a post-hoc subgroup analysis on women aged 35–40 years (*Munné et al., 2019*). Ninety-seven out of 474 (20.5%) consenting participants in this age group failed to achieve at least two blastocysts by day 6. They were therefore not randomized or enrolled. Among study participants with at least two blastocysts the aneuploidy rate was higher in the ARA population (61.9%) compared with women less than 35 years old (49.3%). More women in the ARA subgroup had no euploid embryos available to transfer (17.2%) compared with women under 35 years old (8.9%). The subgroup analysis found a higher OPR with PGT-A when analysed per embryo transfer (50.8% versus 37.2%, $P = 0.035$) but not when analysed per intention-to-treat (41.1% versus 35.7%, $P = 0.35$). Reported miscarriage rates

TABLE 1 SUMMARY OF FINDINGS FOR IVF WITH PGT-A COMPARED WITH IVF ALONE FOR PATIENTS WITH INFERTILITY WITH TWO OR MORE BLASTOCYSTS

Outcomes	Anticipated absolute effects (95% CI)*		Relative effect (95% CI)	No. of participants (studies)	Certainty of the evidence (GRADE)	References
	Risk with IVF alone	Risk with IVF with PGT-A				
Implantation rate assessed with: +GS or +FH / total no. of embryos transferred	55 per 100	66 per 100 (61–73)	RR 1.22 (1.12–1.33)	1308 (5 RCT)	⊕⊕⊕⊕ High[a]	(*Forman et al., 2013*; *Munné et al., 2019*; *Scott et al., 2013*; *Yang et al., 2012*, *2013*)
Early pregnancy loss per clinical pregnancy	19 per 100	12 per 100 (9–18)	RR 0.66 (0.46–0.95)	692 (5 RCT)	⊕⊕⊕O Moderate[a,b,c]	(*Forman et al., 2013*; *Munné et al., 2019*; *Scott et al., 2013*; *Yang et al., 2013*, *2012*)
Clinical pregnancy per embryo transfer	62 per 100	66 per 100 (60–72)	RR 1.06 (0.97–1.16)	1086 (5 RCT)	⊕⊕⊕O Moderate[a]	(*Forman et al., 2013*; *Munné et al., 2019*; *Scott et al., 2013*; *Yang et al., 2013*, *2012*)
Ongoing pregnancy or delivery per embryo transfer	51 per 100	59 per 100 (53–66)	RR 1.15 (1.03–1.28)	1086 (6 RCT)	⊕⊕⊕O Moderate[a]	(*Forman et al., 2013*, 2014; *Munné et al., 2019*; *Scott et al., 2013*; *Yang et al., 2013*, *2012*)
Ongoing pregnancy or delivery per primary embryo transfer	52 per 100	59 per 100 (53–66)	RR 1.15 (1.03–1.28)	1018 (4 RCT)	⊕⊕⊕O Moderate[a]	(*Forman et al., 2013*; *Munné et al., 2019*; *Scott et al., 2013*; *Yang et al., 2012*)
Cumulative delivery per oocyte retrieval	63 per 100	76 per 100 (53–100)	RR 1.21 (0.86–1.72)	103 (1 RCT)	⊕OOO Very low[a,d,e]	(*Yang et al., 2013*)

* The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

GRADE Working Group grades of evidence:

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a] Studies skewed heavily towards young, good-prognosis patients.

[b] Some studies have shown benefit while others have not.

[c] Comprehensive chromosome screening platforms differed between studies.

[d] Includes at most one additional frozen embryo transfer.

[e] Single study with small sample size.

CI, confidence interval; FH, fetal heartbeat; GRADE, Grading of Recommendations Assessment, Development and Evaluation; GS, gestational sac; PGT-A, preimplantation genetic testing for aneuploidies RCT, randomized controlled trial; RR: risk ratio.

were subject to the same definitional concerns as described in the section above. When defined as the number of EPL divided by the number of clinical pregnancies, the rates were not statistically different in the two arms (13.7% in the PGT-A arm and 22.9% in the control arm, $P = 0.16$).

Summary statements:

3. In women aged 35–40 years with at least two blastocysts available, a large multicentre RCT has shown that PGT-A improves implantation rates and OPR per primary transfer; however, when analysed per intention-to-treat there was no improvement in OPR or miscarriage rates.

4. To date, there have been no RCT of PGT-A with trophectoderm biopsy that have examined the time to viable pregnancy in women over age 35 years.

Recommendations:

4. In patients aged 35–40 years undergoing IVF with at least two blastocysts, clinicians may consider the use of PGT-A to improve the OPR per embryo transfer.

Strength: weak.

Quality of evidence: low (TABLE 2).

5. In patients aged 35–40 years undergoing IVF with at least two blastocysts, there is insufficient evidence for the use of PGT-A to reduce the risk of EPL.

Strength: weak.

Quality of evidence: low (TABLE 2).

PGT-A and RPL

IVF with PGT-A is a proposed treatment to prevent miscarriage in patients with RPL. As many patients with RPL do not have concomitant infertility, an alternative to IVF with PGT-A in this population is expectant management. There are no prospective randomized trials that compare PGT-A using CCS with expectant management for karyotypically normal patients with RPL. The only retrospective study to compare PGT-A with expectant management exclusively in the RPL population followed 300 patients with two or more pregnancy losses and normal karyotypes (*Murugappan et al., 2016*). In the expectant management arm, there were 202 6-month windows of attempted natural conception, which resulted in a clinical pregnancy rate of 51% and a pregnancy loss rate of 24%. In the PGT-A arm, there were 168 retrievals resulting in 128 transfers (of which 30 were subsequent FET from the same cycle start) of an average of 1.4 embryos per transfer. Among all patients in the intention-to-treat PGT-A arm, the authors calculated 198 pregnancy attempts. The PGT-A arm had a clinical pregnancy rate of 44% and a pregnancy loss rate of 20%. This was not statistically different from the control arm. Of note, 22.6% of IVF cycles in the treatment arm did not undergo PGT-A due to low embryo yield or poor quality. In a subgroup analysis of the 100 cases that had a euploid embryo transfer, the clinical pregnancy rate was higher than in the controls (72% versus 51%, $P = 0.0008$) but the pregnancy loss rate was not significantly different (14% versus 24%, $P = 0.12$). Importantly, the median time to pregnancy was longer in the PGT-A arm than in the control arm (6.5 months versus 3.0 months). This study has been criticized for its lack of randomization, poor follow-through to PGT-A in the treatment arm, and arbitrary choice of a 6-month window in the control arm (*Rienzi et al., 2017*).

Hodes Wertz and colleagues published a case series of PGT-A results in patients who had a history of two or more unexplained pregnancy losses at less than 20 weeks, normal karyotypes and no uterine or endocrine abnormalities (*Hodes-Wertz et al., 2012*). Patients both with and without concomitant infertility were included. There were 94 cycles that underwent trophectoderm biopsy followed by aCGH-based PGT-A. Eighteen of those cycles had no euploid embryos to transfer. Because of missing or incomplete data, transfer and outcome data were available for 66 cycles. The implantation rate was 50.5% (49/97 embryos), the clinical pregnancy rate 63.6% (42/66 transfers) and the EPL (or termed miscarriage, spontaneous abortion) rate was 4.7% (2/43 pregnancies with implantation). This study did not contain a control group; however, the authors noted that their EPL rates were much lower than expected (i.e. without PGT-A) according to both the frequently cited study by Brigham and colleagues (*Brigham et al., 1999*) and data from the Society for

TABLE 2 SUMMARY OF FINDINGS FOR IVF WITH PGT-A COMPARED WITH IVF ALONE FOR INFERTILITY WITH ADVANCED REPRODUCTIVE AGE

Outcomes	Anticipated absolute effects (95% CI)*		Relative effect (95% CI)	No. of participants (studies)	Certainty of the evidence (GRADE)	References
	Risk with IVF alone	Risk with IVF with PGT-A				
Ongoing pregnancy per embryo transfer	35 per 100	51 per 100 (39–67)	RR 1.45 (1.10–1.91)	276 (1 RCT)	⊕⊕OO Low[a,b,c]	(*Munné et al., 2019*)
Early pregnancy loss per clinical pregnancy	23 per 100	14 per 100 (7–28)	RR 0.60 (0.29–1.23)	143 (1 RCT)	⊕⊕OO Low[a,b,c]	(*Munné et al., 2019*)

* The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

GRADE Working Group grades of evidence:

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a] Post-hoc subgroup analysis.

[b] Does not include any women over 40 years old.

[c] Small sample size.

CI, confidence interval; GRADE, Grading of Recommendations Assessment, Development and Evaluation; PGT-A, preimplantation genetic testing for aneuploidies RCT, randomized controlled trial; RR: risk ratio.

TABLE 3 SUMMARY OF FINDINGS FOR IVF WITH PGT-A COMPARED WITH EXPECTANT MANAGEMENT FOR PATIENTS WITH RECURRENT PREGNANCY LOSS

Outcomes	Anticipated absolute effects (95% CI)*		Relative effect (95% CI)	No. of participants (studies)	Certainty of the evidence (GRADE)	References
	Risk with expectant management	Risk with IVF with PGT-A				
Live birth rate	337 per 1000	318 per 1000 (240–421)	RR 0.0.95 (0.71–1.25)	400 (1 observational study)	⊕OOO Very low[a,b,c]	(*Murugappan et al., 2016*)
Early pregnancy loss rate	240 per 1000	205 per 1000 (120–349)	RR 0.85 (0.50–1.45)	192 (1 observational study)	⊕OOO Very low[a,b,c]	(*Murugappan et al., 2016*)
Clinical pregnancy rate	515 per 1000	444 per 1000 (362–546)	RR 0.86 (0.70–1.06)	400 (1 observational study)	⊕OOO Very low[a,b,c]	(*Murugappan et al., 2016*)

* The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

GRADE Working Group grades of evidence:

High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a] Age in the PGT-A arm was higher than that in the expectant management arm at baseline.

[b] Rationale for length of follow-up in the expectant management arm and the number of cycle attempts in PGT-A arm not clear.

[c] Retrospective study.

CI, confidence interval; GRADE, Grading of Recommendations Assessment, Development and Evaluation; PGT-A, preimplantation genetic testing for aneuploidies RCT, randomized controlled trial; RR: risk ratio.

Assisted Reproduction Technology in the USA (*Hodes-Wertz et al., 2012*).

Recommendations:

6. In patients with RPL, there is insufficient evidence to recommend PGT-A over expectant management to improve LBR.

Strength: weak.

Quality of evidence: very low (TABLE 3).

7. In patients with RPL, there is insufficient evidence to recommend PGT-A to decrease EPL rates.

Strength: weak.

Quality of evidence: very low (TABLE 3).

8. In patients with RPL, there is insufficient evidence to recommend PGT-A to decrease time to live birth.

Strength: weak.

Quality of evidence: very low (expert opinion).

PGT-A and recurrent implantation failure

The use of PGT-A in patients with recurrent implantation failure is reviewed in a separate CFAS guideline entitled *Recurrent Implantation Failure* (*Shaulov et al., 2020*).

LIMITATIONS OF PGT-A

Potential sources of error with PGT-A

PGT-A involves sampling a few cells from an embryo, amplifying a small amount of extracted DNA, and analysing the chromosomal content using one of several available CCS platforms. Human error or handling issues with the biopsy, technical limitations of amplification and CCS, and the intrinsic potential of an embryo to exhibit mosaicism can all lead to test error or misdiagnosis (TABLE 4).

Risk of false positives and false negatives

As PGT-A is a screening test, it is associated with a risk of false positives and false negatives. A false-positive PGT-A result is defined as a biopsy result falsely classified as aneuploid in the setting of a euploid embryo. A false-negative PGT-A result is defined as a biopsy result falsely classified as euploid in the setting of a truly aneuploid embryo. Unlike many other screening tests, however, it is technically challenging to estimate the false-positive and false-negative rates for PGT-A. This unique challenge is due to a lack of a clinical gold standard against which to compare PGT-A results. In practice, presumed euploid embryos are transferred. If a clinical pregnancy results, genetic testing may be undertaken to confirm the genetic status of the pregnancy; however, if no pregnancy or a biochemical pregnancy results, there is no way to confirm that that embryo was in fact euploid. This makes it impossible in clinical practice to ascertain the true extent of false negatives. Conversely, the vast majority of presumed aneuploid embryos are discarded rather than

TABLE 4 SOURCES OF ERROR WITH PGT

Error category	Examples
Human error	Mislabelling Misinterpretation of results/report Transfer of wrong embryo
Technical factors	DNA contamination DNA amplification error Comprehensive chromosome screening platform used Biopsy technique
Intrinsic embryo factors	Mosaicism
Other	Natural conception around time of embryo transfer

PGT, preimplantation genetic testing.

Sources: *Munné et al., 2017*; *Wilton et al., 2009*.

transferred, and therefore their potential to lead to a euploid live birth is not known.

A few clinical studies have attempted to determine the accuracy of PGT-A. The only prospective study to transfer embryos without disclosing the biopsy result found that 3 of 46 (6.5%) PGT-A-screened aneuploid blastocysts resulted in healthy live births (*Scott et al., 2012*). Of note, this study used SNP-based CCS and all embryos also underwent two polar body biopsies in addition to a trophectoderm biopsy, somewhat limiting the applicability of this study. In-vitro studies that have compared the results of trophectoderm biopsy to inner cell mass (ICM) or whole-embryo testing have found variable rates of concordance (80–100%), although the studies have been small (5–52 embryos) and varied in their methods (*Fragouli et al., 2008*; *Huang et al., 2017*, *2019*; *Johnson et al., 2010*; *Liu et al., 2012*; *Orvieto, 2016*). Of note, the study by Huang and colleagues compared trophectoderm biopsy with whole-embryo CCS and found that false-positive results (9 of 18 euploid embryos tested, 50%) were much more common than false-negative results (0 of 32 embryos tested).

Two relatively large retrospective studies have compared the ploidy status of products of conception (POC) and ongoing pregnancies after PGT-A results in an attempt to estimate the risk of an aneuploid pregnancy despite PGT-A-screened euploid embryo transfer. The first examined 3168 transfers of 4974 presumably euploid blastocysts as determined by qPCR-based CCS (*Werner et al., 2014*). Approximately half of the 238 gestational sacs that did not progress to delivery had cytogenetic analysis of POC. The authors documented 10 cases (10/238 = 4.2%) of aneuploid POC – seven after first trimester losses and three in ongoing second-trimester gestations. From this they calculated an error rate of 0.21% per embryo. The second study reviewed 520 transfers of 579 presumably euploid blastocysts as determined by aCGH-based CCS (*Tiegs et al., 2016*). Seventeen of 41 cases of pregnancy loss had cytogenetic analysis of POC. Three aneuploid pregnancies were diagnosed on POC testing (3/17 = 17.6%), and two sex chromosome discrepancies were found in ongoing pregnancies. From this the authors calculated an error rate of 0.9% per embryo transferred. However, these studies do not capture an accurate false-negative rate because they are based on the small subset of cases that miscarry and have POC testing. It is not possible to confirm the ploidy status of screened euploid embryos that fail to implant. Additionally, comprehensive genetic testing of liveborn infants after euploid embryo transfers has not been performed to confirm their euploid chromosomal complement.

Summary statements:

5. The precise false-negative and false-positive rates of PGT-A are difficult to calculate in clinical practice.

6. False-negative PGT-A results can occur as shown by the fact that aneuploid conceptions have been documented in POC and ongoing pregnancies after the transfer of screened euploid embryos.

7. False-positive PGT-A results can occur as shown by the fact that embryos initially screened aneuploid by trophectoderm biopsy have been found to be euploid when re-examined with ICM or whole-embryo testing.

Mosaicism

Embryos are classified by PGT-A as euploid (all cells carry a normal complement of chromosomes), aneuploid (all cells carry an abnormal complement of chromosomes), segmental aneuploid (all cells have a portion of a chromosome duplicated or missing), mosaic (some cells are euploid and some are aneuploid), segmental mosaic (some cells are euploid and some are segmental aneuploid) or inconclusive (failure of DNA amplification or analysis). Embryonic mosaicism arises from mitotic errors that occur post-fertilization. The relative proportion of aneuploid to euploid cells depends on the stage of cleavage at which the error occurs. For instance, an error that takes place at an earlier mitotic cleavage may result in a higher proportion of aneuploid cells (high-level mosaicism) in the resultant embryo, whereas an error at a later mitotic cleavage may result in a lower proportion of aneuploid cells (low-level mosaicism) (*Munné et al., 1994*).

The true incidence of mosaicism in blastocyst embryos is difficult to determine partly because the detection rate depends on the CCS platform used. For example, aCGH can only detect mosaicism if more than 40–50% of the biopsied cells are aneuploid (*Mamas et al., 2012*). Recently, NGS has become the preferred platform of many providers due to reductions in cost, high accuracy and increased dynamic range (*Sachdev et al., 2017*). NGS can detect lower levels of mosaicism than other platforms. In one study using an in-vitro model where euploid and aneuploid cell lines were mixed in known proportions, NGS could detect mosaicism when only 17% of the cells were aneuploid (*Goodrich et al., 2016*). Another factor affecting the rates of mosaicism reported is the variation in mosaicism classification. Some laboratories use lower stringency for classification (i.e. report mosaicism if 20–80% of cells in a biopsy are aneuploid), and others use higher stringency (i.e. report mosaicism if 30–70% of cells are aneuploid). Some laboratories do not report mosaicism at all, considering any biopsy with greater than 40–50% abnormal cells aneuploid and anything below that threshold euploid (*Kim et al., 2018*; *Mamas et al., 2012*).

With an increased ability to detect mosaicism come challenges related to its interpretation. A primary concern is the diagnostic accuracy of a mosaic result. With trophectoderm biopsy, 5–10 cells are removed from the blastocyst; however, it is possible that the degree of mosaicism in these cells may not be representative of the remainder of the embryo (*Vera-Rodriguez and Rubio, 2017*). The remainder of the embryo may have a completely different proportion of euploid or aneuploid cells from the biopsy depending on the distribution of these cells, and the location of the biopsy can influence not only the detection, but also the level of mosaicism reported. Acknowledging this intrinsic sampling limitation of PGT-A, there have been several studies examining the reproducibility of mosaicism reported by NGS. One study performed multiple ICM and trophectoderm re-biopsies on 43 blastocysts previously diagnosed as mosaic and found that 18/43 (42%) had a euploid ICM and 5/42 (12%) were euploid on all rebiopsies (*Garrisi et al., 2016*). A similar study analysed three trophectoderm rebiopsies and the ICM of 16 previously diagnosed mosaic embryos and confirmed mosaicism in only 50% (*Popovic et al., 2018*). The results of these concordance studies depend on the specific criteria for

mosaicism classification, as using lower stringency (i.e. 20–80%) to classify mosaic embryos could lead to more discordance between rebiopsies and the ICM than using higher stringency (i.e. 30–70%).

While the diagnostic accuracy of a single trophectoderm biopsy may be limited, neglecting to report on mosaicism runs the risk of overdiagnosing embryos as aneuploid and discarding potentially viable embryos. Indeed, there is accumulating evidence from multiple observational studies that the transfer of mosaic embryos can result in ongoing pregnancies and healthy live births (*Fragouli et al., 2017*; *Greco et al., 2015*; *Lledó et al., 2017*; *Santiago Munné et al., 2017*; *Victor et al., 2019*; *Zhang et al., 2019*; *Zore et al., 2018*). The opposite, misdiagnosing mosaic embryos as euploid and transferring them resulting in mosaic or abnormal pregnancies, is also possible. In fact, this may be the reason that some PGT-A-screened euploid embryos miscarry. One study used NGS to re-analyse archived DNA from blastocysts previously diagnosed by aCGH as euploid that resulted in EPL, and found that 31.6% were in fact mosaic (*Maxwell et al., 2016*).

In the absence of available euploid embryos after IVF with PGT-A, many patients and physicians struggle with the decision of whether to transfer available mosaic embryos. A survey of 405 IVF clinics in the USA reported that only 36.1% of clinics that carry out PGT-A receive mosaicism data on their reports, and of these clinics 42.9% have transferred a mosaic embryo (*Kim et al., 2018*). Quantifying the outcomes of mosaic embryo transfer has been challenging not only due to the above issues with diagnostic accuracy and thresholds, but also because gold standard randomized studies comparing mosaic with unscreened or euploid embryo transfer cannot be performed due to ethical reasons. Several observational studies have attempted to compare implantation, pregnancy and miscarriage rates of mosaic embryos with those of euploid controls. The results of these studies have been heterogeneous, with a few reporting lower implantation rate, OPR and higher EPL rates with mosaic embryos (*Fragouli et al., 2017*; *Santiago Munné et al., 2017*; *Zore et al., 2018*), and others not showing a significant difference in pregnancy or EPL rates (*Lledó et al., 2017*; *Zhang et al., 2019*). One underpowered study specifically compared low-level mosaic embryos (20–40% abnormal cells) with high-level mosaic embryos (40–80% abnormal cells) and found ongoing implantation rates of 56% in low-level mosaic embryos versus 22% in high-level mosaic embryos ($P = 0.142$) (*Santiago Munné et al., 2017*).

However, when interpreting studies on level of mosaicism, one must be cognisant of the fact that the proportion of aneuploid cells in a single trophectoderm biopsy may not be representative of the entire embryo. The risks of mosaic embryo transfer have also been inadequately studied but, to date, there have been no reports of aneuploid births after mosaic embryo transfer. Recently, the first case of mosaicism detected in a baby born after known mosaic embryo transfer was published, in which the transfer of an embryo with 35% mosaicism of monosomy 2 resulted in a pregnancy with amniocentesis demonstrating a reciprocal (mosaic trisomy 2) result (*Kahraman et al., 2020*). The healthy baby was born at term with no evidence of growth restriction, and a peripheral blood karyotype showed 2% mosaicism of monosomy 2. More postnatal clinical and genetic studies are needed worldwide to better understand the outcomes of mosaic embryo transfers.

Summary statements:

8. The true incidence of embryonic mosaicism is difficult to estimate due to sampling bias, variations in detection rates with different CCS platforms, and different reporting thresholds.

9. Live births have been reported after the transfer of mosaic embryos.

10. The clinical experience with mosaicism is evolving and additional research on pregnancy outcomes and disposition is necessary.

INFORMED CONSENT

It can be challenging for patients to fully understand the complexities of PGT-A. As with any other medical intervention or treatment, it is essential that patients are fully aware of the risks and benefits and provide free and informed consent before proceeding. Ideally, this is best achieved through a counselling appointment with a certified genetic counsellor. Due to the limited number of certified genetic counsellors, particularly those with expertise in PGT, this counselling may be performed by other members of the healthcare team with appropriate training and experience. PGT-A counselling should culminate with all intended parents providing written informed consent for the procedure.

Key points to cover during a PGT-A counselling session are presented as a checklist in TABLE 5.

Recommendation:

9. PGT-A should be undertaken only after thorough counselling and the provision of written informed consent from patients.

FUTURE DIRECTIONS

There are significant deficiencies in the existing literature on PGT-A. Further research is required to better understand the potential benefits, risks and limitations of the technology. First, existing RCT with trophectoderm biopsy have been limited to patients with multiple blastocysts available to biopsy and have focused on reporting pregnancy outcomes per embryo transfer. To appreciate the pragmatic outcomes of PGT-A, future studies are encouraged to randomize patients at cycle start or retrieval and to report the CLBR per cycle started, the time to pregnancy, the chance of having no embryos to biopsy, and the chance of having no euploid embryos to transfer. Longer term studies on obstetric, neonatal and paediatric outcomes after embryo biopsy and PGT-A are also needed to rule out potential downstream risks. Prenatal screening algorithms for pregnancies conceived after euploid embryo transfer are important given the potential risk of false negatives but remain to be clearly established. Cost-effectiveness studies on PGT-A are also lacking and accurate studies on the economics of PGT-A are needed to help with patient decision-making.

To address limitations of the technology such as diagnostic accuracy and mosaicism, more studies are also warranted. More fulsome studies correlating PGT-A results to non-invasive prenatal testing, chorionic villus sampling and amniocentesis results would

TABLE 5 PGT-A COUNSELLING CHECKLIST

Key points
PGT-A currently uses trophectoderm biopsy to screen the number of chromosomes of an embryo
PGT-A is meant to help select the most suitable embryo for transfer
PGT-A may reduce the number of embryos available for transfer
PGT-A does not increase the cumulative live birth rate per cycle started
PGT-A may require that all embryos are frozen while awaiting the results (i.e. there is no fresh embryo transfer)
In some cases PGT-A improves the implantation rate and may reduce the risk of early pregnancy loss
Cost of PGT-A
Risks
Risk of no embryos suitable to biopsy
Risk of damaging the embryo at the time of biopsy
Risk of biopsy transport
Risk of no result or indeterminate result
Risk of an incorrect result (false positive or false negative)
Chance of mosaicism
Criteria for transfer of a mosaic embryo
Risk of no suitable embryo to transfer
Risk of embryo(s) not surviving the biopsy, cryopreservation and thawing process

PGT-A, preimplantation genetic testing for aneuploidies.

improve the estimation of false-negative rates and help guide prenatal testing recommendations after PGT-A. With regards to mosaic embryo transfer, larger scale follow-up studies on pregnancy outcomes need to be performed to better quantify the implantation potential of these embryos and the risks of transfer. Previous observational studies have compared the pregnancy outcomes of mosaic embryos with euploid controls, but a more clinically relevant control group would be unscreened embryos. Surveys and qualitative studies on patients facing the decision to transfer mosaic embryos are needed. Using these data, decision aids may be constructed to help patients decide whether or not to transfer mosaic embryos.

CONCLUSIONS

PGT-A is an adjunct to IVF that may be offered to patients to assist with the selection of the best embryo for transfer. In patients with two or more blastocysts available for biopsy, PGT-A can improve the likelihood that a transferred embryo will lead to a viable pregnancy. Offering PGT-A with eSET reduces the risk of multiple pregnancy, may improve implantation rates and may decrease the risk of EPL per embryo transfer in select populations at higher risk of aneuploidy. Studies that support the use of PGT-A have largely included good-prognosis patients with multiple blastocysts available. The current literature is deficient in examining the use of PGT-A in poor-prognosis patients, who may have difficulty producing blastocysts for testing. Furthermore, there is a paucity of evidence on the effect of PGT-A on the critical outcome of CLBR per cycle started. The current data do not support the universal use of PGT-A for all patients undergoing IVF. Future research should also consider broader issues such as economic costs, patient satisfaction, correlation with prenatal genetic testing, and long-term follow-up studies on the health of children born after PGT. Other technical issues confounding the field include diagnostic inaccuracy and mosaicism. Patient counselling and informed consent before undertaking PGT-A should acknowledge the known limitations of the technology and gaps in knowledge.

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