ANZJOG

DOI: 10.1111/ajo.12960

OPINION

Preimplantation genetic testing for aneuploidy (PGT-A): The biology, the technology and the clinical outcomes

Hayden Anthony Homer[1,2,3]

[1]Christopher Chen Oocyte Biology Research Laboratory, UQ Centre for Clinical Research, The University of Queensland, Brisbane, Queensland, Australia

[2]Reproductive Endocrinology and Infertility Clinic, Royal Brisbane & Women's Hospital, Brisbane, Queensland, Australia

[3]Queensland Fertility Group, Brisbane, Queensland, Australia

Correspondence: Professor Hayden Anthony Homer, Christopher Chen Oocyte Biology Research Laboratory, UQ Centre for Clinical Research, The University of Queensland, Building 71/918, Royal Brisbane & Women's Hospital Campus, Herston, QLD 4029, Australia.
Email: h.homer@uq.edu.au

Conflicts of Interest: The author report no conflicts of interest.

Received: 29 October 2018;
Accepted: 2 January 2019

Preimplantation genetic testing for aneuploidy (PGT-A) seeks to identify preimplantation embryos with a normal chromosome complement (euploid) during *in vitro* fertilisation (IVF). By sifting out embryos with abnormal chromosome numbers (aneuploid), PGT-A should theoretically improve pregnancy success. However, earlier versions of PGT-A were ineffective, and in some cases, detrimental, due to biopsy-induced trauma and because the technology at the time could analyse only a fraction of all chromosomes. More recently, the emergence of technologies enabling all chromosomes to be analysed and a switch to less traumatic blastocyst-stage biopsy have seen widespread uptake of PGT-A. Assessing the full impact of blastocyst biopsy PGT-A requires consideration of multiple factors, including embryonic mosaicism, sensitivity of the technological platform used, embryo loss during long-term *in vitro* culture, embryo cryopreservation and inter-clinic variability in expertise. Significantly, there hasn't yet been an appropriately designed randomised controlled trial (RCT) of blastocyst biopsy PGT-A analysed by intention-to-treat that accounts for all these parameters on a per-cycle basis. The three RCTs reporting benefits studied outcomes on a per-embryo transfer basis were small and underpowered and demonstrated benefits for a very select sub-group of good prognosis patients. The liberal use of this very expensive IVF add-on for other patient populations has not yet been shown to be effective, or indeed, without harm.

KEYWORDS

aneuploidy, embryo, *in vitro* fertilisation, mosaicism, next generation sequencing, PGT-A

THE TECHNOLOGY: NEW AND IMPROVED PGT-A

Embryonic aneuploidy, most often arising from meiotic errors in oocytes, is the major cause of early pregnancy wastage.[1,2] While the hypothesis that pregnancy success should increase by screening out aneuploid embryos is therefore entirely plausible, the previous iteration of preimplantation genetic testing for aneuploidy (PGT-A: so-called PGS 1.0) was unsuccessful.[3] Meta-analysis of 11 randomised controlled trials (RCTs) failed to demonstrate any benefit for PGS 1.0 and indeed, found that it was detrimental for older women.[4]

PGS 1.0 had shortcomings,[3,5] for instance, it involved removal of 1–2 cells from 6–8-cell cleavage-stage embryos, leading to the loss of a large proportion of cell mass (Fig. 1). In one study, implantation rates of 53% for non-biopsied cleavage-stage embryos decreased to 31% post-biopsy.[6] Furthermore,

by testing for less than half of all 24 chromosomes using fluorescence *in situ* hybridisation (FISH), PGS 1.0 missed many abnormalities.

Biopsy for PGT-A can also be performed at the blastocyst stage[3] or by removing the first and second polar bodies from fertilised eggs (Fig. 1A,B).[5,7,8] However, the latter is rarely used nowadays due to technical/logistic difficulties and high costs.[5,7]

Blastocyst biopsy-PGT-A (hereafter BB-PGT-A; Fig. 1) is currently the most widely used approach accounting for ~90% of PGT-A cycles.[9,10] BB-PGT-A involves: (i) culture of embryos for 5–6 days *in vitro* to the blastocyst stage; (ii) removal of 5–10 cells from the trophectoderm (TE), which forms the placenta and other extraembryonic tissues; and (iii) analysis using comprehensive chromosome screening (CCS) technology capable of analysing all chromosomes. Biopsy of blastocysts is less traumatic than at the cleavage stage since a smaller proportion of total cell mass is sacrificed (Fig. 1C).[6] The expectation was that these modifications would cement PGT-A's place in *in vitro* fertilisation (IVF).

THE BIOLOGY: EMBRYONIC MOSAICISM AND SELF-CORRECTION

Human IVF embryos often contain cell lineages having distinctly different chromosomal makeups, termed mosaicism.[7,11–13] Unlike meiotic errors that are uniformly inherited by all embryonic cells (Fig. 2A,B), mosaicism stems from errors in post-fertilisation mitotic cell divisions that stochastically affect any chromosome and any cell within the embryo (Fig. 2C).

Mosaicism is increasingly considered a normal feature of human embryos,[12] consistent with which, rates do not increase

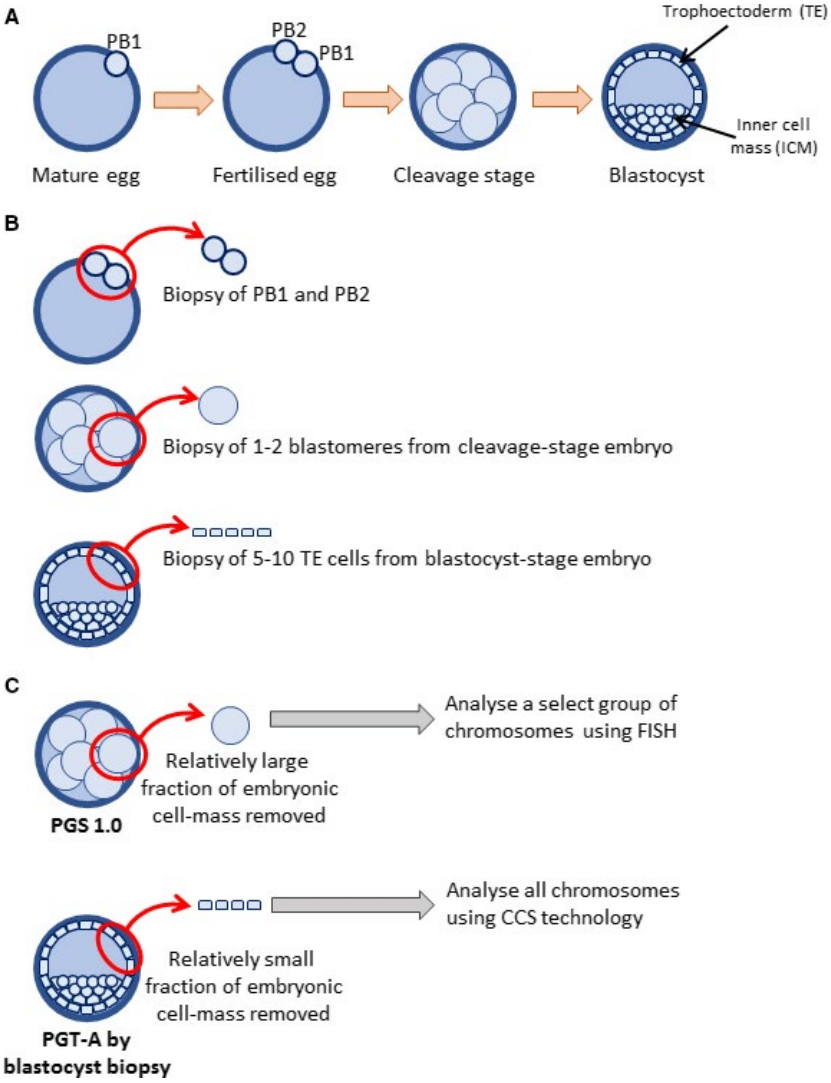

FIGURE 1 Preimplantation embryo development and preimplantation genetic testing for aneuploidy (PGT-A) biopsy approaches. (A) Preimplantation embryo development. Note that development to the blastocyst stage requires 5–6 days of culture in the lab. PB1, first polar body; PB2, second polar body. (B) Three methods for PGT-A biopsy. (C) Comparison of PGS 1.0 and PGT-A by blastocyst biopsy.

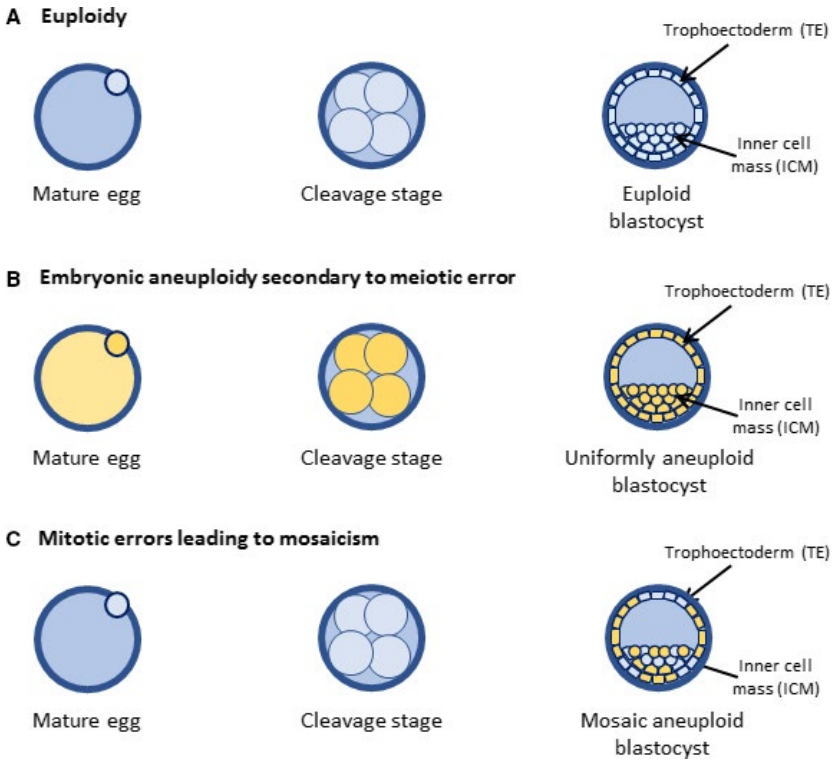

FIGURE 2 Origin of chromosomal errors determines the distribution of aneuploid cells in the blastocyst. (A) No chromosomal errors produce a uniformly euploid blastocyst. (B) A meiotic error arising in the oocyte (yellow cells) is inherited in all subsequent embryonic mitotic divisions resulting in a uniformly aneuploid blastocyst. (C) Mitotic errors arising sporadically in embryonic cells after the cleavage stage produces a mixture of euploid and aneuploid cells within the same blastocyst (mosaic).

with female age[13–16] unlike meiotic errors.[1] Moreover, studies in the mouse model show that mosaic embryos 'self-correct' through clonal depletion of abnormal cells to produce normal offspring.[17] This may also apply to humans since transfer of mosaic embryos during IVF can produce apparently normal offspring,[9,18–21] although it remains unclear whether, or to what extent, mosaicism persists in neonates.

CAN PGT-A DEFINITIVELY ASSIGN ABNORMALITY?

Comprehensive chromosome screening involves molecular genetic approaches including array comparative genomic hybridisation (aCGH) and more recently, next-generation sequencing (NGS).[7,13,22] Biopsied cells are lysed together in a composite sample for analysis. Importantly, therefore, the output from CCS represents the mean of 5–10 cells. While this is effective for uniformly euploid and aneuploid embryos (Fig. 3A,B), it has shortcomings; for example, a sample comprised of four aneuploid cells that are reciprocally trisomic and monosomic for the same chromosome would erroneously generate a normal euploid result (Fig. 4).[16]

CCS platforms have differing abilities for detecting mosaicism – NGS can detect low levels (20%) of mosaicism whereas aCGH (once commonly used) can detect mosaicism only if present at higher levels (>40–50%).[7,13,22] The two platforms can therefore generate different results for the same sample, for instance, 37% of stored TE biopsies initially diagnosed as euploid by aCGH were found to be abnormal when re-analysed using NGS.[23]

Since any cell within an embryo can mis-segregate chromosomes, an infinite variety of mosaic permutations is possible such that aneuploid cells may be confined to the TE or to the inner cell mass (ICM) (Fig. 3C,D) or be dispersed between the two (Fig. 3E). It is therefore impossible to extrapolate with certainty from a small TE sample the make-up of the remainder of the embryo (Fig. 3C–E). The inadequacy of a single 5–10 cell biopsy is supported by theoretical mathematical modelling, which estimated that at least 27 cells would be needed for reasonable predictive power.[24]

One concern, therefore, especially for patients with few embryos, is mistakenly discarding usable embryos because abnormalities identified in the TE are not replicated in the ICM (Fig. 3C). Recently, after analysing disaggregated blastocysts using NGS, 31% were found to have a euploid ICM despite TE abnormalities, the majority of which were mosaic defects.[16] Reassuringly, another recent paper that focused on blastocysts diagnosed as aneuploid on TE biopsy (euploid and mosaic embryos excluded) found high concordance rates in the ICM for whole chromosome aneuploidies

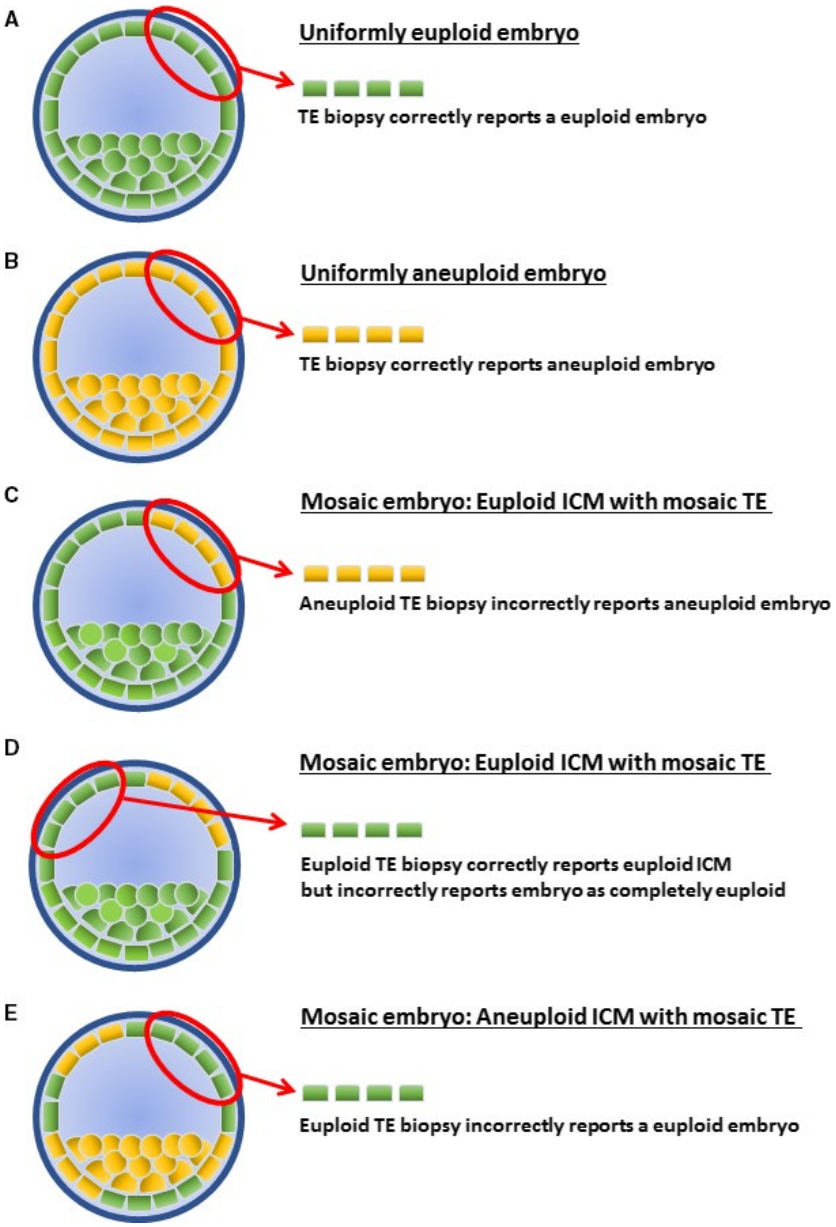

FIGURE 3 Effect of embryonic mosaicism on diagnostic outcomes following blastocyst biopsy. (A and B) Trophectoderm (TE) biopsy from uniformly euploid (A) and uniformly aneuploid (B) embryos accurately diagnose the status of the inner cell mass (ICM). (C) Biopsy of an isolated abnormal segment within the TE of a mosaic embryo correctly determines that the biopsied cells are abnormal but incorrectly diagnoses the ICM as abnormal. (D) Biopsy of another region of the same embryo in (C) correctly identifies biopsied cells and ICM as normal. (E) Biopsy of a normal segment within the TE of a mosaic embryo correctly identifies that the biopsied cells are abnormal but incorrectly diagnoses the ICM as normal.

(97%, 90 of 93) but not for segmental aneuploidies (43%, three of seven).[25]

Mosaicism would be less concerning if overall rates were extremely low in blastocysts. Earlier non-NGS techniques estimated that only ~6% of blastocysts might be mosaic.[11] However, more recent reports paint a different picture. One study found a 30.1% mosaicism rate in TE biopsies from 1547 blastocysts[15] similar to the 28% rate identified in over 2000 biopsies in another study.[26] Analyses of multiple biopsies from the same embryo identified mosaicism rates of 50% (5/10),[27] 37.5% (3/8),[28] 44% (16/36),[29] 34.5% (20/58)[16] and 14% (4/29).[30] Collectively, therefore, based on multiple-biopsy analyses, blastocyst mosaicism rates range from 14% to 50% with a mean of 36%, much higher than previously thought. However, if uniform whole chromosome aneuploidy is identified on TE biopsy, there is a high likelihood (>95%) that the ICM is also aneuploid.[25] Despite this high likelihood, TE aneuploidy does not guarantee uniform embryonic aneuploidy as three of 93 embryos with aneuploid TEs had either mosaic or euploid ICMs.[25]

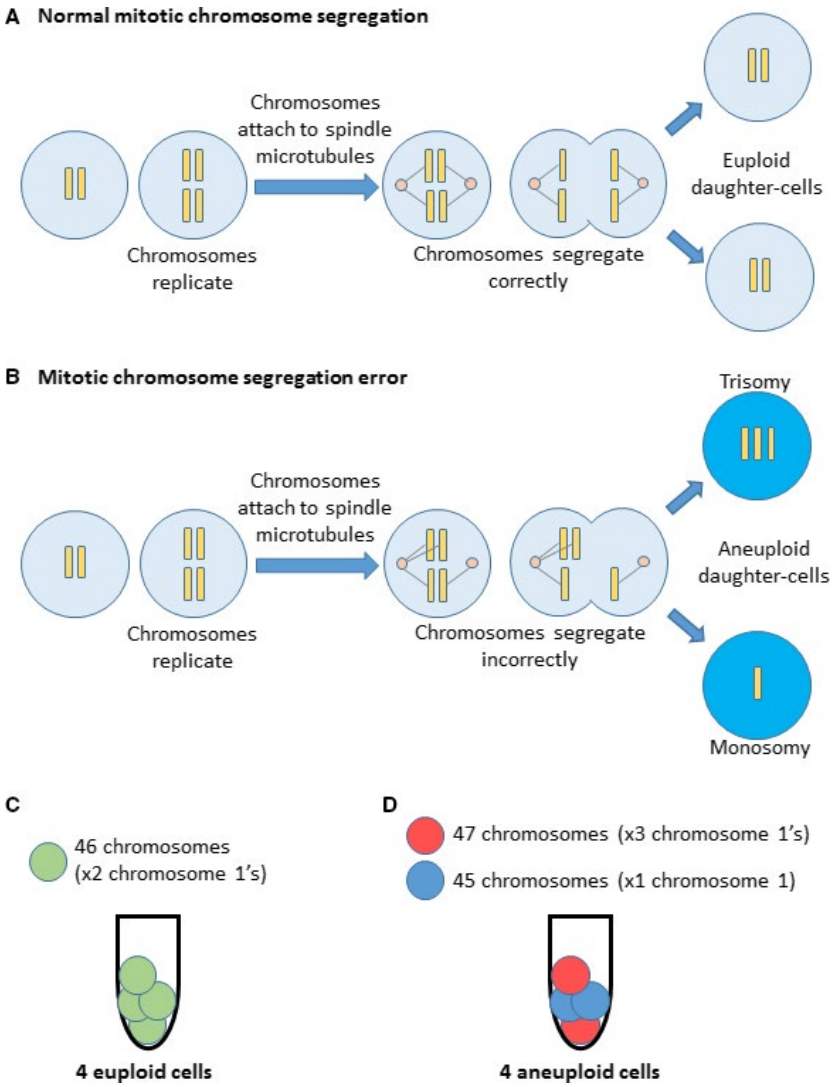

FIGURE 4 Comprehensive chromosome screening (CCS) technologies measure the total number of chromosomes present in the composite sample of biopsied cells. (A) Accurate mitotic chromosome segregation producing euploid daughter cells. (B) Mitotic chromosome mis-segregation of one chromosome leads to aneuploid daughter cells that are reciprocally monosomic and trisomic for that chromosome. (C and D) CCS will produce the same 'euploid' result for a composite sample of four euploid cells each of which contain the correct number of copies of chromosome 1 (green; C) as it would for a merged sample of four aneuploid cells comprised of two cells with an extra copy of chromosome 1 (trisomy 1; red; D) and two cells with one less copy of chromosome 1 (monosomy 1; blue; D). Note that chromosome 1 is used purely for illustrative purposes. See (B) for a schematic explanation of the mechanism by which, mitotic chromosome mis-segregation leads to reciprocal aneuploidies in daughter cells.

BLASTOCYST-BIOPSY PGT-A IN CLINICAL PRACTICE: ANALYSIS BY EMBRYO TRANSFER

Given the widespread adoption of PGT-A into current IVF practice – 40% or more of cycles in the USA are estimated to incorporate PGT-A[10] – it is perhaps surprising that in the aftermath of PGS 1.0, few RCTs have to-date evaluated BB-PGT-A. Three small and underpowered RCTs (89, 72 and 55 patients in the treatment groups) have been published in full[31–33] along with a larger multi-centre RCT published in abstract form (274 treatment patients).[34]

Two RCTs were conducted in the same high-volume PGT-A clinic and involved transfer of two embryos per patient.[31,32] Both involved good prognosis patients (5–8 blastocysts per patient) who underwent randomisation if they had two or more blastocysts. Unlike most clinics, this one has an on-site genetics laboratory enabling CCS results to be obtained fast enough after day 5 biopsy to facilitate a fresh transfer the following day. PGT-A patients had higher sustained implantation rates after one embryo transfer (66.4% vs 47.9%; $P = 0.001$).[32]

The other RCT from this group found that fresh transfer of a single euploid blastocyst was non-inferior to fresh transfer of two unbiopsied blastocysts.[31] Multiple pregnancies were eliminated by single embryo transfer (SET) compared to a staggering 53% multiples rate following double embryo transfer (DET).[31]

Two RCTs have compared outcomes after SET.[33,34] The first analysed pregnancy success following a single fresh embryo transfer in 103 (55 PGT-A and 48 control) good prognosis patients (mean age 31 years, ~19 oocytes and eight blastocysts per patient) treated at two centres.[33] Ongoing pregnancy rates following fresh embryo transfer were higher following PGT-A (69.1% vs 41.7%; $P = 0.009$).[33]

More recently, the results of the multi-centre Single Embryo TrAnsfeR (STAR) trial were reported in abstract format. This RCT was undertaken across four countries, nine genetic laboratories and 34 clinical sites.[34] A total of 588 patients having a mean age of 34 years were randomised on days 5/6 of embryo culture and blastocysts were vitrified following biopsy. In stark contrast to the foregoing trials, there was no difference in the primary outcome of ongoing pregnancy rate following transfer of single thawed embryos (49.6% vs 45.9%; $P = 0.3369$).[34] A *post hoc* sub-group analysis pointed to a benefit with PGT-A for older patients (35–40 years; 50.8% vs 37.2%; $P = 0.0349$).[34]

The multi-centre/multi-clinic nature of the STAR trial along with incorporation of embryo freezing provides insight into how PGT-A might perform more generally and seriously questions whether the advantages seen in high-performing clinics undertaking a fresh embryo transfer and with good prognosis patients can be extrapolated more broadly, a concern also raised by the American Society for Reproductive Medicine.[35] Indeed, the STAR trial's lead author suggested 'that some less experienced centres may be losing embryos through the process'.[10]

PGT-A OUTCOMES BY INTENTION-TO-TREAT ANALYSES

The outcome of greatest significance to patients is the overall chance of live birth per round of ovarian stimulation. When incorporating BB-PGT-A, the answer to this question needs to factor in a key risk not accounted for in the above trials: the likelihood that no or few embryos develop to the blastocyst stage, especially when few eggs are collected. By only randomising patients already known to have blastocysts and/or incorporating good prognosis patients with high likelihood of having blastocysts, existing trials do not address this issue.[31–34] This is a critical consideration since highly effective embryo vitrification allows virtually all usable embryos to be transferred in fresh and subsequent frozen-thawed cycles, whereas with BB-PGT-A, embryos that could have been transferred on days 2/3 might be lost during an additional 2–4 days of *in vitro* culture or from biopsy trauma and/or misdiagnosis due to mosaicism. Therefore, proper evaluation of BB-PGT-A requires an intention-to-treat design that factors in cumulative outcomes following transfer of all suitable embryos from one stimulation round in fresh and frozen cycles as well as the possibility that no embryos survive to blastocyst stage.[36–39] To date, no such RCT has evaluated BB-PGT-A and all trials have excluded patients with low egg yields. However, two RCTs have used intention-to-treat methods to evaluate PGT-A by cleavage-stage and polar body biopsy.

The first involved women of advanced maternal age (38–41 years old).[40] Interestingly, despite evidence that cleavage-stage biopsy is traumatic,[6] these researchers used cleavage-stage biopsy due to their clinic's extensive 15-year experience with the technique. Fresh embryo transfers were performed 2 days later at the blastocyst stage after biopsy results had become available. Live-birth rates per transfer were higher with PGT-A but there were no overall differences when cumulative outcomes for the entire cycle (including frozen embryos) were considered (37% vs 33%).[40] Patients in the PGT-A arm had fewer embryos transferred per cycle (1.3 vs 1.8; $P < 0.0001$), required fewer transfers (1.3 vs 1.0; $P < 0.0001$) and experienced fewer miscarriages (39% vs 2.7%; $P = 0.0007$).[40] Intention-to-treat analyses typically evaluate outcomes from a *single* round of ovarian stimulation. In contrast, in this trial, patients needed to have at least five mature eggs and if not achieved after one round of stimulation/oocyte retrieval, women were required to undergo a second round; indeed, those refusing a second round were eliminated from the trial.[40] Ensuring a minimum number of oocytes mitigates against the risk of not having any suitable embryos and cannot therefore reflect the real-life per-cycle performance of PGT-A.

More recently, results of the ESTEEM (the ESHRE Study into the Evaluation of oocyte Euploidy by Microarray analysis) trial based on polar body biopsy (PBB) were reported.[41] ESTEEM was a multi-centre trial involving nine centres in seven countries in Europe and Israel having experience in PBB.[8,41] ESTEEM involved 396 patients aged 36–40 years with good ovarian reserve and tested whether PGT-A by PBB would improve the chances of a live birth within 1 year. Both groups achieved the same pregnancy rate (24%) and PGT-A did not shorten the time to pregnancy.[41] However, PGT-A reduced miscarriage from 14% to 7% ($P = 0.02$) and increased the number of SETs (19% vs 56%; $P < 0.001$) with a corresponding trend toward reduced twins.[41]

These trials confirm that overall chances of live birth do not improve with PGT-A, which is not surprising since PGT-A does not increase embryo potential. However, PGT-A may yield secondary benefits such as reduced miscarriages.

RELEVANCE OF AVAILABLE DATA TO AUSTRALIAN PRACTICE

PGT-A provides confidence to undertake SET in programs that routinely undertake DET, thereby reducing multiple pregnancies.[31,41] However, the most recent Australian data show that 87.7% of all cycles and 93% of cycles in women under 35 involved SET.[42] Therefore, in Australia, deciding whether to perform DET or SET is not a dilemma that clinicians face.

The three RCTs demonstrating benefit with BB-PGT-A transferred fresh embryos,[31–33] but in most clinics around the world including Australia, fresh PGT-A transfers are very infrequent after blastocyst biopsy since biopsy samples are shipped to external genetic labs, necessitating that embryos be frozen. When embryo

freezing is involved, in the same time it would take to undergo a PGT-A FET cycle, both a fresh and a frozen SET could be undertaken without PGT-A. This means that for a comparable good prognosis group as in the SET RCT, ~42% of patients would miss out on pregnancy from a fresh transfer.[33] Moreover, the expected cumulative pregnancy rate from a fresh and a frozen transfer (~66%) would be comparable to a single FET of a tested embryo (69.1%).[33]

The STAR trial is arguably the most informative in the context of PGT-A in Australia since it involved BB-PGT-A and frozen SET. As elaborated above, this trial did not demonstrate any overall benefit for PGT-A.[34]

INTER-LABORATORY VARIABILITY AND FINANCIAL COSTS

PGT-A-related embryo trauma is critically dependent upon laboratory proficiency, firstly, for long-term culture to the blastocyst stage and, secondly, for the technically exacting biopsy itself. Lab quality could also impact the risk of iatrogenic embryonic aneuploidy.[43] For PGT-A to deliver a benefit, it was estimated that lab-induced embryo loss rates should be <10%, and that rates ≥30% negate any potential benefit unless aneuploidy rates exceed 60%.[10] In line with this, the Chairman of the 2018 congress of the Preimplantation Genetic Diagnosis International Society (PGDIS), Santiago Munne, stated that 'standardisation of embryo biopsy is clearly needed to democratise PGT to the masses'.[10]

Since PGT-A doesn't increase overall chances of live birth,[40,41] an important consideration is whether the high financial costs might be justified by possible secondary benefits (eg reduced emotional distress associated with miscarriage), noting that such benefits have not yet been demonstrated for BB-PGT-A. It has recently been estimated that for a typical 36–37-year-old patient in the USA, PGT-A would cost $30 000 USD for a 90% chance of having one euploid embryo.[44] For older patients with low ovarian reserve, costs escalated to staggering amounts ranging from $200 000 to $400 000 USD.[44] The Spanish RCT found that including PGT-A increased costs required to achieve a live birth without increasing overall chances of having a live birth.[40] A theoretical model that evaluated cost-effectiveness based on UK practice concluded that adding PGT-A was unlikely to benefit most women under 40 years.[45]

CONCLUSIONS

The PGDIS recommended that only validated NGS platforms should be used for PGT-A.[46] However, because of the unpredictable nature of mosaicism, even the most sensitive technology cannot unequivocally predict the status of the ICM based on 5–10 TE cells.[16,24]

BB-PGT-A in good prognosis patients with multiple blastocysts and in expert PGT-A labs could increase pregnancy rates in a single fresh cycle and reduce DETs without compromising success.[31–33] However, there are no RCTs of BB-PGT-A analysed by intention-to-treat. At present, the only trial of BB-PGT-A that encompasses all possibilities on a per-stimulated-cycle basis is a hypothetical one, the outcomes for which disfavour BB-PGT-A.[38]

An important unanswered question pertains to use of mosaic embryos given that many can generate healthy pregnancies.[9,18,20,21] A grading system has been proposed for prioritising their utilisation based on severity of abnormality[47] but the rationale has been vigorously challenged.[36,48,49]

Finally, the STAR trial illustrates that extrapolating results from experienced labs to widespread practice could be very misleading.[10,34] The American Society for Reproductive Medicine recently concluded that 'The value of PGS/PGT-A as a screening test for IVF patients has yet to be determined'.[35]

ACKNOWLEDGEMENTS

HH is supported by the Christopher Chen Endowment Fund and NHMRC Grants APP1078134 and APP1103689.

REFERENCES

1. Greaney J, Wei Z, Homer H. Regulation of chromosome segregation in oocytes and the cellular basis for female meiotic errors. *Hum Reprod Update* 2018; **24**: 135–161. https://doi.org/10.1093/humupd/dmx035
2. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update* 2002; **8**: 333–343.
3. Geraedts J., Sermon K. Preimplantation genetic screening 2.0: the theory. *Mol Hum Reprod* 2016; **22**: 839–844.
4. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. *Hum Reprod Update* 2011; **17**: 454–466.
5. Harper JC. Preimplantation genetic screening. *J Med Screen* 2018; **25**: 1–5.
6. Scott RT Jr, Upham KM, Forman EJ *et al.* Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril* 2013; **100**: 624–630.
7. Griffin DK, Ogur C. Chromosomal analysis in IVF: just how useful is it? *Reproduction* 2018; **156**(1): F29–F50.
8. Magli MC, Montag M, Köster M *et al.* Polar body array CGH for prediction of the status of the corresponding oocyte. Part II: technical aspects. *Hum Reprod* 2011; **26**: 3181–3185.
9. Fragouli E, Alfarawati S, Spath K *et al.* Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid-aneuploid blastocysts. *Hum Genet* 2017; **136**: 805–819.
10. Munne S. Status of preimplantation genetic testing and embryo selection. *Reprod Biomed Online* 2018; **37**: 393–396.
11. Capalbo A, Rienzi L. Mosaicism between trophectoderm and inner cell mass. *Fertil Steril* 2017; **107**: 1098–1106.
12. McCoy RC. Mosaicism in preimplantation human embryos: when chromosomal abnormalities are the norm. *Trends Genet* 2017; **33**: 448–463.
13. Munne S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. *Fertil Steril* 2017; **107**: 1085–1091.

14. McCoy RC, Demko ZP, Ryan A *et al.* Evidence of selection against complex mitotic-origin aneuploidy during preimplantation development. *PLoS Genet* 2015; **11**: e1005601.
15. Nakhuda G, Jing C, Butler R *et al.* Frequencies of chromosome-specific mosaicisms in trophoectoderm biopsies detected by next-generation sequencing. *Fertil Steril* 2018; **109**: 857–865.
16. Popovic M, Dheedene A, Christodoulou C *et al.* Chromosomal mosaicism in human blastocysts: the ultimate challenge of preimplantation genetic testing? *Hum Reprod* 2018; **33**: 1342–1354.
17. Bolton H, Graham SJ, Van der Aa N *et al.* Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. *Nat Commun* 2016; **7**: 11165.
18. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. *N Engl J Med* 2015; **373**: 2089–2090.
19. Lledo B, Morales R, Ortiz JA *et al.* Implantation potential of mosaic embryos. *Syst Biol Reprod Med* 2017; **63**: 206–208.
20. Munne S, Blazek J, Large M *et al.* Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with the use of high-resolution next-generation sequencing. *Fertil Steril* 2017; **108**: 62–71.e68.
21. Spinella F, Fiorentino F, Biricik A *et al.* Extent of chromosomal mosaicism influences the clinical outcome of *in vitro* fertilization treatments. *Fertil Steril* 2018; **109**: 77–83.
22. Brezina PR, Anchan R, Kearns WG. Preimplantation genetic testing for aneuploidy: what technology should you use and what are the differences? *J Assist Reprod Genet* 2016; **33**: 823–832.
23. Maxwell SM, Colls P, Hodes-Wertz B *et al.* Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. *Fertil Steril* 2016; **106**: 1414–1419.e1415.
24. Gleicher N, Metzger J, Croft G *et al.* A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use. *Reprod Biol Endocrinol* 2017; **15**: 33.
25. Victor AR, Griffin DK, Brake AJ *et al.* Assessment of aneuploidy concordance between clinical trophectoderm biopsy and blastocyst. *Hum Reprod* 2018; **34**: 181–192.
26. Sachdev NM, Maxwell SM, Ribustello L *et al.* The high rate of abnormal embryos in donor cycles is reflected in donor oocyte pregnancy outcomes. *Fertil Steril* 2016; **106**: e150–e151.
27. Gleicher N, Vidali A, Braverman J *et al.* Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos. *Reprod Biol Endocrinol* 2016; **14**: 54.
28. Orvieto R, Shuly Y, Brengauz M, Feldman B. Should preimplantation genetic screening be implemented to routine clinical practice? *Gynecol Endocrinol* 2016; **32**: 506–508.
29. Tortoriello DV, Dayal M, Beyhan Z *et al.* Reanalysis of human blastocysts with different molecular genetic screening platforms reveals significant discordance in ploidy status. *J Assist Reprod Genet* 2016; **33**: 1467–1471.
30. Chuang TH, Hsieh JY, Lee MJ *et al.* Concordance between different trophectoderm biopsy sites and the inner cell mass of chromosomal composition measured with a next-generation sequencing platform. *Mol Hum Reprod* 2018; **24**: 593–601.
31. Forman EJ, Hong KH, Ferry KM *et al. In vitro* fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril* 2013; **100**: 100–107.e101.
32. Scott RT Jr, Upham KM, Forman EJ *et al.* Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril* 2013; **100**: 697–703.
33. Yang Z, Liu J, Collins GS *et al.* Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. *Mol Cytogenet* 2012; **5**: 24.
34. Munne S, Kaplan B, Frattarelli JL *et al.* Global multicenter randomized controlled trial comparing single embryo transfer with embryo selected by preimplantation genetic screening using next-generation sequencing versus morphologic assessment. *Fertil Steril* 2017; **108**: e19.
35. Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. *Fertil Steril* 2018; **109**: 429–436.
36. Braude P. The emperor still looks naked. *Reprod Biomed Online* 2018; **37**: 133–135.
37. Gleicher N, Orvieto R. Is the hypothesis of preimplantation genetic screening (PGS) still supportable? A review *J Ovarian Res* 2017; **10**: 21.
38. Orvieto R. Preimplantation genetic screening- the required RCT that has not yet been carried out. *Reprod Biol Endocrinol* 2016; **14**: 35.
39. Schattman GL. Chromosomal mosaicism in human preimplantation embryos: another fact that cannot be ignored. *Fertil Steril* 2018; **109**: 54–55.
40. Rubio C, Bellver J, Rodrigo L *et al.* In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. *Fertil Steril* 2017; **107**: 1122–1129.
41. Verpoest W, Staessen C, Bossuyt PM *et al.* Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. *Hum Reprod* 2018; **33**: 1767–1776.
42. Fitzgerald O, Paul RC, Harris K, Chambers GM. *Assisted Reproductive Technology in Australia and New Zealand 2016*, Sydney: National Perinatal Epidemiology and Statistics Unit, the University of New South Wales Sydney, 2018.
43. Munne S, Alikani M, Ribustello L *et al.* Euploidy rates in donor egg cycles significantly differ between fertility centers. *Hum Reprod* 2017; **32**: 743–749.
44. Goldman RH, Racowsky C, Farland LV *et al.* The cost of a euploid embryo identified from preimplantation genetic testing for aneuploidy (PGT-A): a counseling tool. *J Assist Reprod Genet* 2018; **35**: 1641–1650.
45. Scriven PN. Towards a better understanding of preimplantation genetic screening for aneuploidy: insights from a virtual trial for women under the age of 40 when transferring embryos one at a time. *Reprod Biol Endocrinol* 2017; **15**: 49.
46. Newsletter P. *PGDIS Position Statement on Chromosome Mosaicism and Preimplantation Aneuploidy Testing at the Blastocyst Stage, Chicago, Illinois*, 2016. Available from URL: http://www.pgdis.org/docs/newsletter_071816.html.
47. Grati FR, Gallazzi G, Branca L *et al.* An evidence-based scoring system for prioritizing mosaic aneuploid embryos following preimplantation genetic screening. *Reprod Biomed Online* 2018; **36**: 442–449.
48. Gleicher N, Kushnir VA, Barad DH. How PGS/PGT-A laboratories succeeded in losing all credibility. *Reprod Biomed Online* 2018; **37**: 242–245.
49. Murtinger M, Wirleitner B, Schuff M. Scoring of mosaic embryos after preimplantation genetic testing: a rollercoaster ride between fear, hope and embryo wastage. *Reprod Biomed Online* 2018; **37**: 120–121.