Pre-Implantation Genetic Screening Techniques: Implications for Clinical Prenatal Diagnosis

Stefan C. Kane\(^a, b\) Elissa Willats\(^c\) Sammya Bezerra Maia e Holanda Moura\(^a, g\) Jonathan Hyett\(^f\) Fabrício da Silva Costa\(^b, d, e\)

\(^a\)Pregnancy Research Centre, Department of Maternal Fetal Medicine, and \(^b\)The University of Melbourne, Department of Obstetrics and Gynaecology, The Royal Women’s Hospital, Parkville, Vic., \(^c\)Monash IVF Pty Ltd, \(^d\)Monash Ultrasound for Women, and \(^e\)Perinatal Services, Monash Medical Centre, Clayton, Vic., and \(^f\)RPA Women and Babies, Royal Prince Alfred Hospital, Sydney, N.S.W., Australia; \(^g\)University of Fortaleza, Fortaleza, Brazil

Key Words
Pre-implantation genetic testing · Pre-implantation genetic screening · Pre-implantation genetic diagnosis · Prenatal diagnosis · Aneuploidy

Abstract
Chromosomal aneuploidy is responsible for a significant proportion of pregnancy failures, whether conceived naturally or through in vitro fertilization (IVF). In an effort to improve the success rate of IVF, screening embryos for aneuploidy – or pre-implantation genetic screening (PGS) – has been proposed as a means of ensuring only euploid embryos are selected for transfer. Early PGS approaches were based on fluorescence in situ hybridization testing, and have been shown not to improve live birth rates. Recent developments in genetic testing technologies – such as next-generation sequencing and quantitative polymerase chain reaction, coupled with embryo biopsy at the blastocyst stage – have shown promise in improving IVF outcomes, but they remain to be validated in adequately powered, prospective randomized trials. The extent to which IVF with PGS lowers the a priori risk of aneuploidy in ongoing pregnancies so conceived has been poorly described, rendering it difficult to incorporate the potential benefit of PGS into existing prenatal aneuploidy screening regimens such as cell-free DNA testing or conventional combined nuchal translucency and maternal biochemistry assessment. Further data on the sensitivity and specificity of various forms of molecular PGS testing would improve our understanding of the effectiveness and accuracy of these technologies. This, in addition to further research into methods of risk combination and assessment, would allow us to help our patients make better-informed decisions about whether or not to proceed with invasive diagnostic tests.

Introduction

Compared with other mammalian species, human reproduction is extremely inefficient. A fertile couple who are trying to conceive has only a 25% chance of falling pregnant each menstrual cycle [1]. Even if a successful pregnancy is achieved, fewer than 50% of naturally conceived human embryos are capable of developing to term. While many factors contribute to this high rate of embryo loss, one of the most significant is chromosomal aneu...
Aneuploidy [2]. Cytogenetic research has indicated that aneuploidy is relatively common during pre- and post-implantation development, affecting up to 50% of pre-implantation embryos generated by in vitro fertilization (IVF) [3–5] and 10% of all first-trimester pregnancies [6]. Although most aneuploid conceptuses perish in utero, some survive to term and often present with specific and complex phenotypes including developmental disabilities and intellectual impairment [7].

The high frequency of aneuploid conceptions in humans, and their contribution to pregnancy loss and adverse perinatal outcome, has prompted the development of extensive prenatal and pre-implantation testing programmes. The latter are necessarily performed in the context of assisted reproduction, the success, accessibility, and increasing acceptability of which have led to an increased uptake of these services, with over 4% of births in Australia in 2013 being the product of assisted reproductive technologies [8]. Achieving a successful outcome can, however, be difficult, and many women face significant emotional and financial distress caused by repeated IVF cycle or pregnancy failure. For older women, more than 60% of autologous embryos may be aneuploid, conferring a 40% risk of miscarriage after successful conception [9].

A number of techniques have been developed to identify euploid embryos prior to implantation, in the belief that doing so will increase the proportion of successful IVF cycles with elective single-embryo transfer and minimize the risk of miscarriage, abnormalities at birth, and multiple pregnancy complications [9]. This technology is called pre-implantation genetic screening (PGS), which today can assess all 23 pairs of chromosomes prior to the selection and transfer of an embryo. The primary indications for this screening tool include advanced maternal age [5, 10–17], repeated implantation failure [16, 18, 19], recurrent miscarriage [20, 21], and severe male factor infertility [22], although many clinicians are now offering this testing to all patients undergoing IVF [23].

There are few published data about the accuracy of currently available PGS tests. As a result, many obstetricians not directly involved in IVF are unlikely to be aware of the potential impact of different biopsy and molecular testing techniques on the effectiveness of PGS – and the residual risk of aneuploidy in pregnancies so conceived. Such information is vital for adequate antenatal counselling, especially in circumstances of an abnormal result on cell-free DNA aneuploidy screening or conventional combined first-trimester aneuploidy screening (nuchal translucency and maternal serum β-hCG and PAPP-A), or the finding of a structural anomaly at the time of the 12- or 20-week scan, despite the transfer of an embryo that was apparently normal at PGS.

Herein we review:
- current biopsy techniques for PGS;
- laboratory genetic testing techniques used in PGS; and
- the interplay between PGS and prenatal aneuploidy screening and diagnosis.

Biopsy Techniques for PGS

PGS can potentially be performed on three different cell types during pre-implantation development: polar bodies (PBs) from the oocyte and zygote, blastomere(s) from cleavage-stage embryos (fig. 1), or trophectoderm cells obtained from the blastocyst (fig. 2). Each of these three approaches has advantages and disadvantages in terms of the accuracy of genetic diagnosis and its potential impact on the IVF process [24], as outlined in table 1.

A final factor of relevance to selecting the optimal time to perform embryo biopsy is that of embryo ‘self-correction’, the phenomenon by which an apparently aneuploid embryo undergoes a corrective process to achieve a diploid state. Embryo ‘self-correction’ was initially proposed based on PGS observations that a significant proportion of embryos diagnosed as aneuploid at the cleavage stage are capable of developing into apparently euploid blastocysts [25–27]. While such observations may be due to laboratory misdiagnosis or mosaicism, there have been reports of embryos with mosaic trisomy undergoing early...
Table 1. Advantages and disadvantages of different biopsy techniques for PGS

<table>
<thead>
<tr>
<th>Technique</th>
<th>Detail</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB biopsy</td>
<td>Earliest stage at which cells can be obtained</td>
<td>Provides the earliest specimen for PGS, maximizing time for genetic analysis in the case of fresh embryo transfer</td>
<td>Assumes that embryonic aneuploidy results from non-disjunction in maternal meiosis [32, 66], whereas in fact this may only account for 66% [86], leaving one-third of aneuploidies undetected</td>
</tr>
<tr>
<td></td>
<td>Based on the premise that the chromosomal make-up of PBs reflects that of the oocyte [83]</td>
<td>Potentially less invasive, as PBs do not make a physical contribution to the embryo [84]</td>
<td>May not be cost-effective: 2 PBs are sampled per oocyte, not all of which will develop into embryos for transfer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No apparent effect on fertilization rates or further embryo development [85]</td>
<td></td>
</tr>
<tr>
<td>Blastomere biopsy</td>
<td>Biopsied 3 days after fertilization, at the 6- to 10-cell stage [87, 88]</td>
<td>Sampled from the embryo itself, therefore more representative of its chromosomal make-up than PB biopsy</td>
<td>Requires removal of a significant proportion of the embryo’s cells, which – in addition to the disruption to the cellular junctions – may adversely impact developmental viability [90, 91] and implantation rates [91]</td>
</tr>
<tr>
<td></td>
<td>Removal of blastomeres is facilitated by incubation in Ca^{2+}/ Mg^{2+}-free medium, which disrupts cellular junctions [89]</td>
<td></td>
<td>Biopsied cell/s may not be representative of the whole embryo, especially given that up to 29% of pre-implantation embryos are mosaic [32, 92–94]</td>
</tr>
<tr>
<td>Blastocyst biopsy</td>
<td>Hole drilled in the zona pellucida on day 3, then cultured to blastocyst stage (days 5–6)</td>
<td>Randomized controlled trial evidence of no adverse impact on embryo implantation potential [32, 95]</td>
<td>The late stage at which the biopsy is performed limits the time for genetic analysis prior to fresh embryo transfer [32, 69]; as a consequence, many laboratories vitrify embryos after biopsy [91, 97]; this vitrification strategy may result in higher pregnancy rates anyway [98]</td>
</tr>
<tr>
<td></td>
<td>Some trophoderm cells (that will develop into the placenta) herniate through the hole in the zona pellucida and are removed for diagnosis, leaving the inner cell mass intact</td>
<td>Allows more cells to be taken for analysis</td>
<td>Chromosomal make-up of the trophoderm may not be representative of the inner cell mass in up to 4% of cases [83, 88]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Greater efficiency associated with delaying biopsy to days 5–6, as many abnormal embryos lack the developmental capacity to grow to the blastocyst stage [88, 96]</td>
<td>Mosaicism is as prevalent as on day 3 [83]</td>
</tr>
</tbody>
</table>

Fig. 2. Blastocyst biopsy (days 5–6).
postzygotic mitotic loss of the trisomic chromosome, or ‘trisomic rescue’, resulting in a diploid fetus [28] and potentially healthy outcome [29]. However, it is also possible that the two remaining chromosomes will be derived from the one parent (i.e. uniparental disomy), which can result in imprinting errors or homozygosity for a recessive condition [30]. Furthermore, placentae in gestations that have undergone trisomic rescue demonstrate confined placental mosaicism, which predisposes to placental dysfunction and its sequelae such as fetal growth restriction [31], and can lead to misleading results following chorionic villus sampling or cell-free fetal DNA testing in maternal serum [28]. Based on an analysis of uniparental disomy frequency in newborns, it has been concluded that embryo self-correction is a rare event, occurring with a frequency of less than 1% [32, 33]. Therefore, the possibility of embryo ‘self-correction’ should not be given significant weight when selecting the optimal time for embryo biopsy.

Laboratory Techniques for Pre-Implantation Genetic Testing

Once material has been obtained for genetic analysis, one of several screening protocols can be employed. As PGS involves the analysis of only one or a few cells, it is imperative that these screening strategies are rapid, highly reliable, and accurate, as well as capable of providing the maximum amount of genetic information about the biopsy sample. In addition to these requirements, the screening test should ideally be able to be completed within a minimal time frame so as to enable a fresh embryo transfer if so desired.

Fluorescence in situ Hybridization

Fluorescence in situ hybridization (FISH) was the first technique used to pioneer PGS [34]. Initially employed in 1992 as a means of sex selection for the prevention of X-linked recessive diseases [35], FISH was subsequently adopted worldwide for the analysis of chromosome copy numbers and some structural chromosomal abnormalities in both metaphase chromosomes and interphase nuclei (fig. 3) [36–38].

Despite its promise of improved clinical outcomes, randomized controlled trials performed over the past 10 years have found that FISH-based PGS does not improve live birth rates [16, 23, 39–47], potentially on account of the limited number of chromosomes assessed [48], the early stage of the biopsy, and mosaicism [49–51].

FISH has now been superseded by a range of more advanced molecular genetic tests that are capable of simultaneously analysing all 23 pairs of chromosomes with an apparently greater accuracy and consequently lower rate of misdiagnosis [52, 53]. It is anticipated that these new comprehensive screening tests, coupled with a shift towards performing embryo biopsy at the blastocyst stage, will allow the potential of PGS to be realized [54, 55]. Numerous different testing platforms are now used for comprehensive chromosome screening, including microarray-based comparative genomic hybridization (aCGH), single nucleotide polymorphism microarrays (SNP arrays), quantitative polymerase chain reaction (qPCR),

---

Fig. 3. FISH-based PGS testing for chromosomes X (blue), Y (yellow), 13 (red), 18 (aqua), and 21 (green) (colours refer to the online version only). Based on single-cell analysis, these embryos were diagnosed as male with no abnormalities detected (a), male with trisomy 18 (b), and female with trisomy 21 (c).
Table 2. Technical aspects of different genetic tests used in PGS

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCGH</td>
<td>Based upon the same principles as traditional CGH to metaphase chromosomes [99–102], aCGH is dependent upon successful whole genome amplification (WGA) of DNA from the biopsied cell(s) in order to generate enough DNA for analysis. Following WGA, the embryonic DNA is fluorescently labelled, denatured, and hybridized to an array platform containing thousands of DNA probes that are specific to each of the human chromosomes. Unbound or non-specifically bound DNA is removed by washing, and a scanning device is used to measure the fluorescence intensity at each of the probes on the array. By comparing the fluorescence intensity of the embryonic sample with that of a control male sample (± control female sample), it is possible to determine the copy number of each chromosome in the biopsied cell(s) [103, 104].</td>
</tr>
<tr>
<td>SNP arrays</td>
<td>As with aCGH, SNP array testing requires successful WGA of DNA from the biopsied cell/s in order to generate enough DNA for analysis. Following WGA, the embryonic DNA is fragmented and hybridized to a SNP array platform, which contains probes for more than 300,000 different SNP sites across the genome. Following hybridization, an extension and staining step is performed. A/T nucleotides at the SNP site are labelled with a red fluorochrome, and G/C nucleotides at the SNP site are labelled with a green fluorochrome. By measuring the intensity of red-to-green fluorescence at each SNP site on the array, it is possible to simultaneously genotype more than 300,000 SNPs in each sample. In some cases, parental DNA samples (or samples from the two biological contributors to the embryo) are also assessed. Because the embryonic chromosomes are derived from the parental chromosomes, this parental SNP information can be used to track the inheritance of chromosomal material from the parents to the embryos. This helps to ‘clean up’ the noisy single-cell microarray data. In this way, many of the errors that are introduced during the WGA procedure (e.g. allele dropout, preferential amplification and amplification failure) can be detected and the data adjusted to factor this in for the final analysis [105].</td>
</tr>
<tr>
<td>qPCR</td>
<td>Involves the pre-amplification of embryonic DNA, followed by a high-order multiplex PCR reaction designed to amplify several loci from each chromosome. With the use of real-time qPCR, each product is quantitated, allowing a comparison across the genome [67].</td>
</tr>
<tr>
<td>NGS (fig. 4)</td>
<td>As with aCGH and SNP arrays, NGS-based PGS requires WGA of the DNA from the biopsied cell/s in order to generate enough DNA for testing. Once amplified, the embryonic DNA is fragmented and tagged with a specific barcode to enable sample tracking. Hundreds of thousands of these small barcoded embryonic DNA fragments (from multiple embryo biopsy samples) are mixed together and sequenced in parallel. Following sequencing, specialized computer software is used to differentiate the unique sample tracking barcodes, thereby enabling the results to be segregated according to embryo biopsy sample. Once segregated, each sequenced fragment from each sample is compared against the reference human genome and aligned with its corresponding chromosome region. The number of aligned sequences along the length of each chromosome is then calculated. Because the number of aligned sequences should be proportional to the copy number present in the original sample, trisomy or monosomy can be confidently identified based on a corresponding increase or decrease in the number of aligned sequences along the length of the chromosome.</td>
</tr>
</tbody>
</table>
and next-generation sequencing (NGS). Each of these tests is discussed in more detail below; their technical aspects are outlined in table 2, while their diagnostic capabilities and associated advantages and disadvantages are summarized in table 3.

### Microarray-Based Comparative Genome Hybridization

aCGH was one of the first techniques used to provide comprehensive PGS of all 23 pairs of chromosomes, and is a technique that is still widely used in clinical practice. aCGH technology has been successfully applied to PBs [56–58], cleavage-stage embryos [56, 57, 59, 60], and blastocysts [56], with published accuracies ranging from 98% following blastomere biopsy [59] to 95% following blastocyst biopsy [56]. In 2011, Geraedts et al. [57] reported a proof-of-principle study that aimed to determine the feasibility and reliability of PGS when performed on PBs. Both first and second PBs were biopsied and analysed using aCGH. Embryos deemed to be aneuploid based on the PB assessment were re-biopsied and tested at the zygote stage to determine concordance of results. In 138/156 cases (88%), complete information was available on both PBs and the corresponding zygotes. In 130/138 of these (94%), the ploidy status of the zygote was concordant with the ploidy status predicted by the PBs. The remaining 8/138 cases (6%) had discordant results. The authors concluded that the ploidy status of the embryo can be predicted with acceptable accuracy by aCGH analysis of both PBs [57].

While initially used to screen at-risk embryos, such as those from patients with known parental chromosomal translocations [56, 60, 61], aCGH has subsequently become more widely applied for PGS in patients with a good prognosis. In 2012, Yang et al. [62] published a randomized pilot study investigating the use of aCGH for PGS in first-time IVF patients with a good prognosis (i.e. maternal age below 35 years, no prior miscarriage, and normal karyotype). The patients were prospectively randomized into one of 2 groups. In the first group (n = 55), embryos were selected for transfer based on morphology assessment...
and aCGH results (embryo biopsy performed at the blastocyst stage). In the second group (n = 48), embryos were selected for transfer based on morphology assessment alone. All patients had a single fresh blastocyst transferred on day 6. The clinical pregnancy rate was significantly higher in the morphology-plus-aCGH group than in the morphology-only group (70.9 vs. 45.8%; p = 0.017), as was the ongoing pregnancy rate at 20 or more weeks of gestation (69.1 vs. 41.7%; p = 0.009). Based on this, the authors concluded that aCGH significantly improves clinical pregnancy rates and reduces miscarriage rates in patients with a good prognosis undergoing fresh embryo transfer cycles.

Yang et al. [63] then extended this study to evaluate whether blastocyst biopsy with aCGH testing prior to cryopreservation could similarly improve pregnancy and implantation outcomes in frozen embryo transfer cycles. A significantly higher implantation rate was observed in the morphology-plus-aCGH group than in the morphology-only group (65.0 vs. 33%; p = 0.038). Although not significant, the morphology-plus-aCGH group also showed a decrease in miscarriage rate compared to the morphology-only group (0 vs. 16.7%; p > 0.05) [63].

While the results of both of these studies are encouraging, the impact of this technology on live birth rates remains to be established, and further randomized controlled trials with a larger sample size are needed to validate these preliminary findings. Further studies are also required to determine whether or not these findings may be replicated in patients with a poor prognosis with decreased ovarian reserve.

**SNP Arrays**

SNP arrays are also commonly used for PGS. A pioneer prospective, randomized, blinded, and paired study compared the accuracy of SNP array testing with FISH; 160 cleavage-stage embryos were analysed, 75 by FISH and 85 by SNP array. The SNP array produced significantly more interpretable results (96%) than FISH (83%) (p < 0.004). In addition, mosaicism was significantly less commonly observed by SNP array (31%) than by FISH (100%) (p < 0.0005) [64].

The accuracy of SNP array PGS was assessed in a prospective, randomized, and blinded study published by Treff et al. [65] in 2010. Aneuploid and euploid cell lines were obtained from a public repository, and blastomeres were obtained from embryos generated by 78 patients undergoing IVF. Single cells extracted from karyotypically defined cell lines provided 99.2% accuracy for individual SNPs, 99.8% accuracy for whole chromosomes, and 98.6% accuracy when applying a quality control threshold for the overall assignment of aneuploidy status. The concordance for more than 80 million SNPs in 335 single blastomeres was 96.5%.

In a subsequent study by Scott et al. [66], 255 embryos were biopsied (113 on day 3 and 142 on days 5–6) and the embryo/s transferred prior to PGS being performed. Following embryo transfer, PGS was performed on the biopsy samples using SNP array technology to determine whether PGS would have diagnosed the embryos as euploid (suitable for transfer) or aneuploid (not suitable for transfer). The patients were monitored after transfer. If a pregnancy was established, a DNA sample was obtained from the conceptus (through cell-free fetal DNA at approx. 9 weeks gestation, or from a buccal cell swab following delivery). Using DNA fingerprinting, it was possible to compare the results from the conceptus with the results of the embryos that were transferred in order to determine which embryo had implanted and sustained development. The results of this were used to determine the predictive value of PGS (i.e. if available prior to transfer, could the PGS result have predicted which embryos would implant and result in a successful pregnancy). Overall, 72/255 embryos (28.2%) resulted in clinical implantation. PGS was found to be highly predictive of clinical outcome: 41% of the embryos that had been diagnosed as euploid implanted successfully, compared with only 4% of the embryos that had been diagnosed as aneuploid [66].

**Quantitative Polymerase Chain Reaction**

qPCR is very effective in the assessment of known single gene defects, and has traditionally been used for pre-implantation genetic diagnosis rather than PGS. In more recent years, however, the technique has been adapted to provide a rapid method of PGS which is able to be completed in just 4 h [67]. One of the first reports investigating the use of qPCR for PGS was published by Treff et al. [67], who performed a prospective, randomized, and blinded study. Their aim was to evaluate whether qPCR could correctly diagnose chromosome copy numbers in 9 karyotypically defined cell lines and 71 discarded blastocysts that had previously undergone PGS using the SNP array. Samples from the 9 cell lines were diagnosed by qPCR with 97.6% accuracy (41/42), increasing to 100% following application of a minimum threshold for concurrence. Based upon analysis of the blastocyst biopsy samples, qPCR was able to confirm the original SNP array PGS result in 70/71 cases (98.6%). Overall, euploidy (n = 37) and aneuploidy (n = 34) were assigned with 100% consistency, highlighting the clinical utility of the qPCR technique [67].
In 2013, Forman et al. [68] performed a randomized controlled trial to assess the impact of PGS by qPCR on obstetric and neonatal outcomes in infertile couples with a maternal age of 42 years and above. The patients were randomized into one of 2 groups when at least 2 embryos had developed sufficiently to allow for blastocyst biopsy. In the first group (n = 89), embryos were biopsied at the blastocyst stage and PGS was performed using qPCR. At the completion of this rapid PGS testing, a single euploid embryo was transferred. In the second group (n = 86), two untested blastocysts were transferred. While the delivery rates following the fresh cycle and up to 1 frozen transfer were very similar between the two groups, the untested 2-embryo transfer group was found to have a significantly higher delivery rates per cycle were also significantly higher in the PGS group than in the control group (84.7 vs. 67.5%; p = 0.01). Finally, the patients randomized into this study were infertile couples with a maternal age or oocyte donor age of 21–42 years who had had no more than 1 prior failed IVF attempt. The patients were randomized into one of two groups. In the first group (n = 72), embryos were biopsied at the blastocyst stage and PGS was performed using qPCR. Euploid embryos were transferred fresh on day 6 of culture. In the second group (i.e. the control group; n = 83), untested embryos were transferred fresh on day 5 of culture. While both groups had excellent outcomes, the PGS group was found to have a significantly higher sustained implantation rate than the control group (66.4 vs. 47.9%; p < 0.001). The delivery rates per cycle were also significantly higher in the PGS group than in the control group (84.7 vs. 67.5%; p = 0.01) [69].

**Next-Generation Sequencing**

The latest technique to be applied to PGS is NGS (fig. 4). Although relatively new to the PGS sphere, NGS is well accepted in the prenatal testing arena, and is the most frequently used method for aneuploidy screening through assessment of cell-free DNA in maternal serum [70].

The potential role for NGS in PGS was assessed by Yin et al. [71], who analysed 38 blastocyst biopsy samples with both SNP arrays and NGS. All 26 euploid embryos as well as 6 embryos with uniform aneuploidies were correctly identified by both SNP array and NGS. Furthermore, NGS also detected all 6 embryos with unbalanced chromosomal translocations, 1 of which was not identified by SNP array.

Fiorentino et al. [72] performed a retrospective study to validate the use of NGS for PGS on single cells. Eighteen karyotypically defined, chromosomally abnormal single cells and 190 whole genome amplification products from single blastomeres (previously analysed using aCGH) were blindly evaluated using NGS. Overall, NGS showed a high level of concordance with established methodologies, with concordance achieved for 207/208 samples (99.5%). In the remaining sample, NGS was found to produce a false-positive call for trisomy 18 (not detected using aCGH, and similarly not detected upon re-analysis using qPCR). However, as this false positive occurred in a biopsy sample exhibiting multiple aneuploidies, the overall concordance of aneuploid embryo call between technologies was 100%.

Based on the success of this initial preclinical validation, Fiorentino et al. [73] performed a prospective trial to assess the clinical potential of NGS when performed in parallel with aCGH on blastocyst biopsy samples. A total of 192 blastocysts from 55 PGS cycles were biopsied and evaluated in a double-blind strategy using both NGS and aCGH. Concordant results were obtained for 191/192 blastocysts (99.5%). Again, the single discordant sample contained several aneuploidies; thus, as such, NGS achieved an overall specificity and sensitivity of 100%. A 62% ongoing implantation rate was achieved, resulting in 30 term deliveries and 31 healthy infants.

Yang et al. [74] performed a randomized clinical study to evaluate the efficiency of NGS for PGS in comparison to aCGH. In this study, 172 patients (mean maternal age 35.2 ± 3.5 years) were randomized into 2 groups. The first group (n = 86) had their blastocysts screened using NGS, while the second group (n = 86) had their blastocysts screened using aCGH. All blastocysts were vitrified after biopsy and pending PGS results. If available, 1 or 2 euploid blastocysts were thawed for transfer in a subsequent frozen embryo transfer cycle. NGS and aCGH resulted in similarly high ongoing pregnancy rates (74.7 vs. 69.2%; p > 0.05) and implantation rates (70.5 vs. 66.2% of embryos transferred; p > 0.05). Based on these results, the authors concluded that NGS was a highly accurate, efficient, and high-throughput technology suitable for use in PGS.
Fig. 4. PGS results obtained following blastocyst biopsy and NGS. The x-axis of each graph depicts the chromosomes (1–22, X, and Y), while the y-axis depicts the chromosome copy number. Samples were diagnosed as euploid male (a), euploid female (b), male with trisomy for chromosome 22 (c), female with trisomy for chromosome 13 (d), male with monosomy for chromosome 16 (e), female with monosomy for chromosome 1 (f), male with monosomy for chromosomes 13 and 21 (g), and female with monosomy for chromosomes 13 and X (h).
The Impact of PGS on Pregnancy Outcomes

The clinical outcome of greatest relevance to subfertile couples undertaking IVF is their likelihood of taking home a healthy live-born infant. Relatively few studies of PGS have this as their primary endpoint, reporting instead on more easily ascertained outcomes such as implantation and pregnancy rates, which are also likely to be more favourable.

As described above, newer testing technologies – coupled with biopsy at the blastocyst rather than cleavage stage of embryonic development [75] – may result in improved pregnancy outcomes, although randomized controlled trial-level evidence in this regard remains scant [76]. Indeed, to date there has only been one such trial – by Scott et al. [69] – which evaluated whether blastocyst biopsy and rapid qPCR-based PGS improves IVF implantation and delivery rates in women with a favourable prognosis. As noted earlier, 84.7% of the cycles in the PGS group resulted in delivery, compared with only 67.5% in the control group (p = 0.01; RR 1.26, 95% CI 1.05–1.50). Two other trials suggested that PGS results in a greater proportion of pregnancies continuing beyond 20 weeks’ gestation [62], or achieves lower multiple pregnancy rates for the same ongoing pregnancy rate [68], but they did not specifically report on the outcome of live birth. In contrast to the findings of the randomized controlled trial [69], a meta-analysis of 3 cohort studies that addressed the impact of PGS on live birth rates found a suggestion of benefit that was not statistically significant (RR 1.35, 95% CI 0.85–2.13) [76].

Thus, the use of PGS in conjunction with biopsy at the blastocyst stage may in fact improve clinically relevant patient outcomes, although further broad-based, adequately powered, prospective clinical studies are required to confirm the role of PGS, particularly in groups without a favourable prognosis. Until then, the true potential benefit or harm of these technologies will remain unknown [23, 77, 78].

The Impact of PGS on Prenatal Screening and Diagnosis

There is very little published on the potential impact of PGS on prenatal screening and diagnosis. The traditional approach to prenatal diagnosis uses a two-tier process, recognizing that diagnostic tests (chorionic villus sampling and amniocentesis) are invasive, risk miscarriage, and therefore have the potential to cause more harm than good if applied universally. Diagnostic tests are, therefore, traditionally reserved for women deemed to be at high risk after screening.

Combined first-trimester screening (including ultrasound assessment of nuchal translucency and the measurement of the serum biochemistry markers β-hCG and PAPP-A) and second-trimester maternal serum screening (using α-fetoprotein, hCG, oestriol, and – on occasion – inhibin) are the most frequently used tests to screen for aneuploidy [79]. Risks are produced by measurement of these parameters and the production of likelihood ratios that reflect gestation-dependent levels seen in euploid and aneuploid pregnancies. The likelihood ratio is then applied to an a priori risk, based on maternal age, gestational age, and a history of previous aneuploidy. One disadvantage of this process is that IVF appears to affect the biochemical constitution of the pregnancy, potentially leading to erroneous calculation of likelihood ratios, increasing false positive rates through screening [23]. This is not an issue for cell-free DNA screening, which is rapidly gaining acceptance as a screening test for common aneuploidies in both high-risk [80] and routine [81] obstetric populations.

Conventional screening programmes aim to identify pregnancies at high risk of trisomies 21, 18, and 13. Risk algorithms for sex chromosome aneuploidy are also available. The inclusion of PGS effectively changes the prevalence of aneuploidy in the prenatal environment and – assuming the original prevalence of aneuploidy was the same in IVF and spontaneous pregnancy cohorts – the sensitivity of the PGS technique can be used to adjust the a priori risk accordingly. The likelihood ratio for aneuploidy generated by either conventional screening or cell-free DNA testing should ideally be applied to the a priori risk adjusted for PGS rather than that generated by the patient’s age and history alone. This would effectively lower the screen positive rate in these screening programmes, given that pregnancies achieved following PGS will by definition have a lower pre-test probability for aneuploidy. Further research is required to define the exact extent to which novel PGS approaches reduce the risk of aneuploidy in ongoing pregnancies, which would allow for an accurate calculation of a priori risks.

The findings of increased nuchal translucency, markedly deranged biochemical parameters, or fetal structural problems in later pregnancy present another dilemma, as these are all now recognized to be associated with atypical aneuploidies as well as with the conventional trisomies for which screening is performed prenatally [82]. In this circumstance, the process of risk assessment and counselling must focus on the potential effectiveness of PGS and
Implications

The development and application of new molecular technologies allowing embryo selection has stimulated further interest in the value and use of PGS in IVF, although their role in improving the live birth rate remains to be confirmed in prospective randomized trials. These techniques appear to be effective in detecting conventional trisomies and other forms of aneuploidy, and therefore reduce the risk of a pregnancy being affected by one of these conditions when a woman attends for prenatal screening.

Our ability to include the value of PGS in calculating prenatal screening risks is dependent first on the recognition that PGS has been performed and second on identification of the type of PGS used. Further data on the sensitivity and specificity of various forms of molecular PGS testing would improve our understanding of the effectiveness and accuracy of these technologies. This, in addition to further research into methods of risk combination and assessment, would allow us to help our patients make better-informed decisions about whether or not to proceed with invasive diagnostic tests.

Acknowledgements

S.C.K. is supported by a Postgraduate Scholarship from the Australian National Health and Medical Research Council. His PhD project is funded by grants from the Research Foundation of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists, and from the Australasian Society for Ultrasound in Medicine.

Statement of Ethics

Ethics approval was not required for the preparation of this review article.

Disclosure Statement

The authors have no conflicting or competing interests to declare.

References


Pre-Implantation Genetic Screening

DOI: 10.1159/000449381

Fetal Diag Thera 2016;49:241–254

251


44 Brezina PR, Kuttel WH: Clinical applications of preimplantation genetic testing. BJM 2015;350:i7611.

Pre-Implantation Genetic Screening

Implications

DOI: 10.1159/000449381

Fetal Diagn Ther 2016;49:241–254

253

Pre-Implantation Genetic Screening

Implications

DOI: 10.1159/000449381

Fetal Diagn Ther 2016;49:241–254

253


90 Cohen J, Wells D, Munné S: Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates. Fertil Steril 2007;87:496–503.