24-chromosome copy number analysis: a comparison of available technologies

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Chromosome aneuploidy, an abnormal number of chromosomes, in human gametes and embryos is a major cause of IVF failure and miscarriage and can result in affected live births. To avoid these outcomes and improve implantation and live birth rates, preimplantation genetic screening aims to identify euploid embryos before transfer but has been restricted to analysis of a limited number of chromosomes. Over the past 15 years, various technologies have been developed that allow copy number analysis of all 23 pairs of chromosomes, 22 autosomes, and the sex chromosomes, or "24-chromosome" copy number analysis in single or small numbers of cells. Herein the pros and cons of these technologies are reviewed and evaluated for their potential as screening or diagnostic tests when used in combination with oocyte or embryo biopsy at different stages. (Fertil Steril® 2013;100:595–602. ©2013 by American Society for Reproductive Medicine.)

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From the earliest years of IVF, it had been suspected that a high incidence of chromosome aneuploidy in human oocytes and embryos might contribute to low implantation and pregnancy rates, and the first attempt to karyotype embryos was reported 30 years ago (1). Only three 8-cell stage embryos were successfully karyotyped out of eleven analyzed, and two were identified as aneuploid. This high incidence of aneuploidy, albeit in a very small sample, clearly alarmed the authors and prompted them to try to reassure clinicians and patients with the statement: "It must be emphasised that over 100 babies have been born following in vitro fertilization without any apparent chromosome abnormality. Chromosome abnormalities of the kind we have found clearly result in early embryonic loss, and probably contribute to the high failure rate after embryo transfer."

Today, with the development of a range of molecular genetic technologies that allow copy number analysis for all 23 pairs of chromosomes, 22 pairs of autosomes, and the sex chromosomes, or "24 chromosomes," in single or small numbers of cells, there is now definitive evidence for the high incidence of abnormal chromosome copy number, or aneuploidy, in both gametes and all stages of preimplantation development. Furthermore, these aneuploidies can arise through gonadal mosaicism, during meiosis (predominantly female meiosis), and in the mitotic cleavage divisions following fertilization up to and including the blastocyst stage (2).

The challenge for embryologists and clinicians remains how to use this knowledge to improve clinical practice. No one would knowingly transfer an aneuploid embryo or, for example, continue with multiple IVF cycles in a patient with a very high incidence of aneuploidy and consequently a low or zero chance of achieving a pregnancy with her own oocytes. On the other hand, any strategy to avoid these scenarios with the use of the available technologies for aneuploidy testing has to balance the benefits of identifying euploid embryos for transfer with the potential costs to the embryo of any invasive biopsy or any false positive and negative test results. The pros and cons of different biopsy methods are reviewed elsewhere in this section. Here, I present an overview of available and emerging technologies for 24-chromosome copy number analysis.

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SCREENING VERSUS DIAGNOSIS

The testing of oocytes and preimplantation embryos for aneuploidy with the aim of improving IVF outcomes, particularly reducing miscarriage rates and increasing live birth rates, is now widely referred to as preimplantation genetic screening (PGS). However, before comparing the different technologies, it is instructive to examine the different expectations for a screening versus a diagnostic test, in the stricter sense of those terms (Table 1).

Quintessentially, a screening test is noninvasive, rapid, and sufficiently low cost for application to all patients to prioritise embryos for transfer. Furthermore, the requirements for accuracy are likely to be less stringent, although false positive results, which may exclude embryos with normal copy number, are arguably more undesirable than false negative results. A good example of such a test is counting the number of pronuclei formed after insemination. Although useful as an early indication of fertilization rate, it was originally intended to avoid the transfer of triploid embryos arising from dispermic fertilization, which is one of the commonest causes of early miscarriage. However, it is well known that in some cases the formation of pronuclei is asynchronous and apparent third pronuclei may simply be empty vesicles. Furthermore, molecular genetic analysis by karyomapping (see later section) has revealed that among embryos identified as normally fertilized with two pronuclei, it is relatively common to find unfertilized parthenogenetically activated haploid or triploid fertilized embryos (unpublished observations). So a routine test, which is universally applied to all IVF cycles, is accepted because of the advantages of monitoring the fertilization rate and the low cost of making the observations, despite the accuracy not being 100%.

Another example of a noninvasive method for embryo selection, which could potentially be used to identify aneuploid embryos, is the use of incubators fitted with time-lapse microscopy allowing detailed morphokinetic analysis of each embryo (3). There have now been several reports of an association of different parameters with aneuploidy (4). However, the effectiveness and accuracy of morphokinetic analysis for identifying aneuploid embryos with only a single aneuploidy versus those with multiple aneuploidies and aneuploidies of different origins has not been established. In principle, it seems unlikely that all aneuploidies could be identified in this way, because many implant and cease development only at later stages of pregnancy.

With a diagnostic test, in contrast, the costs, both financial and to the viability of embryo, of the necessary invasive testing, are still important but secondary to the paramount objective of diagnostic accuracy (Table 1). The requirement of a diagnostic test is a high sensitivity and specificity and in particular a very low incidence of false negative results. So, for example, preimplantation genetic diagnosis (PGD) of a severe single gene defect typically requires the use of multiple highly polymorphic markers specific for the parental chromosomes in the region of the gene combined with mutation detection. Here the aim is to identify two, and only two, chromosomes, one from each parent, with any appropriate combination of unaffected and affected chromosomes. Using this strategy theoretically reduces the chance of misdiagnosing an unaffected embryo to <1 in 1,000. However, any partial or ambiguous results may result in an unaffected embryo not being transferred.

For PGS and 24-chromosome copy number analysis, if the aim is simply to improve IVF rates and reduce miscarriage rates, a noninvasive test with moderate accuracy may be effective. On the other hand, for a patient who has experienced repeated pregnancy loss with karyotypically abnormal conceptuses, the aim is to avoid miscarriage or fetal abnormality and an invasive test with a low false negative rate may be more appropriate. Furthermore, whereas the efficacy of any screening test needs to be evaluated by a randomized controlled trial (RCT) and analysis of clinical pregnancy and live birth rates, the efficacy of a diagnostic test needs to be established by validation of the methodology, follow-up analysis of tested embryos, and monitoring of the pregnancy outcome at birth.

TABLE 1

Screening versus diagnostic testing of chromosome copy number in preimplantation embryos.

Screening	Diagnosis
All patients	Specific indications
Minimally invasive	Invasive
All embryos	Good-quality embryos only
Rapid with fresh transfer	Rapid with fresh transfer, or not time limited with vitrification
High efficiency	Moderate efficiency
Direct or indirect	Direct
Accurate	Highly accurate
Low false negatives acceptable	Tolerate false positives No false negatives
Clinically effective	Validation of diagnostic accuracy
Randomized control trials	
Low cost	Medium to high cost

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24-CHROMOSOME COPY NUMBER ANALYSIS

The simplest and least expensive method for identifying abnormalities of chromosome number is to spread and count stained metaphase chromosomes on glass microscope slides. However, as the original study by Angell et al. (1) demonstrated, the proportion of embryo cells that can be arrested in metaphase by microtubule inhibitors is relatively low and the chromosomes often overlap or are scattered across the slide and can be lost. Furthermore, because the chromosomes are generally short and can not be banded by standard staining methods, the accuracy is reduced further as the pairs of chromosomes cannot be identified. Although there have been many studies of human gametes and embryos with the use of karyotyping, the low efficiency per cell prevents its use for screening purposes. This has led to the search for molecular cytogenetic technologies applicable at the level of single or small numbers of cells, which ideally would avoid the need to arrest cells in metaphase. A range of technologies have been investigated over the past 15 years, including methods which simply aim to count the overall number of

chromosomes, those that identify some or all chromosome pairs, and, more recently, technologies that quantify the amount of chromosomal DNA present or identify each individual chromosome and its parental origin.

The first molecular cytogenetic technique to be applied widely to interphase nuclei spread on slides was fluorescence in situ hybridization (FISH) with a combination of chromosome-specific probes labeled with different fluorochromes. Although the number of probes which could be used in the same hybridization was limited to about five, the first probe set can be washed off the slides and a sequential hybridization performed so that the number can be increased. However, the efficiency of hybridization declines rapidly and the most that can be analyzed with any accuracy is ~12–14 chromosomes. Multicolor FISH typically with 5–9 probes in two sequential hybridizations became the standard methodology for PGS for a number of years. However, RCTs demonstrated a decrease or no improvement in live birth rates per cycle started in a range of indications. This was attributed to a number of factors, including FISH errors caused by overlapping or split signals in single interphase nuclei and particularly the limited number of chromosomes analyzed. Therefore, the focus has now shifted to technologies that allow all 24 chromosomes to be analyzed. However, attempts to do this with FISH-based methods, for example, using short fluorescent oligonucleotide probes in a series of sequential hybridizations, are not sufficiently accurate at the single-cell level (5), and 24-color FISH methods require metaphase chromosomes (6).

COMPARATIVE GENOMIC HYBRIDIZATION

Comparative genomic hybridization (CGH) was originally developed for karyotyping solid tumors, which are difficult to karyotype by conventional methods. The method involves isolating DNA from the test sample and from a karyotypically normal individual and labeling the DNA with red and green fluorochromes. The two labeled DNAs are then cohybridized to a normal metaphase chromosome spread and the intensity of fluorescent labeling with the two probes is analyzed with a microscope fitted with appropriate filters, a sensitive camera, and dedicated software. To adapt the method for use with single cells isolated from cleavage-stage embryos, Wells et al. (7) used degenerate oligonucleotide priming polymerase chain reaction (PCR) to amplify the whole genome of both the test cell and control genomic DNA to avoid artefacts caused by amplification bias. However, accurate quantification requires a long hybridization of ~3 days. Nevertheless, this approach was the first 24-chromosome copy number technology to be used clinically, using a strategy of cleavage-stage biopsy followed by cryopreservation of the biopsied embryos for transfer in a later cycle after completion of the CGH analysis (8).

More recently, conventional CGH has been used successfully for polar body analysis with fresh transfer (9, 10). Furthermore, a fast 12-hour protocol has now been developed which has a resolution high enough to be applicable to not only whole chromosome aneuploidy but also some translocation chromosome imbalance (11, 12).

ARRAY CGH

The principles of microarray-based CGH (array CGH) are the same as for conventional CGH, requiring labeled DNA from both test and control samples, but the labeled DNA is then hybridized to a DNA microarray rather than a metaphase spread. Analysis is performed by scanning and imaging the array and measuring the intensity of both hybridization signals relative to each probe (logR ratio). It was only with the advent of multiple displacement amplification (MDA) for whole-genome amplification, which allows micrograms of DNA to be amplified from single or small numbers of cells, that the use of microarrays could be considered for preimplantation genetics (13, 14). For copy number analysis, however, a PCR library–based whole-genome amplification method is preferable because of reduced amplification bias, which improves the accuracy of ratio detection and reduces variability between probes across each chromosome. For single cell analysis, arrays of ~3,000 large fragments of human DNA cloned in bacteria from loci distributed at ~1-Mb intervals across each chromosome are now available, allowing accurate copy analysis of whole-chromosome copy number as well as partial-chromosome copy number abnormalities of chromosome arms or smaller regions down to a resolution of ~10 Mb. Furthermore, a higher-resolution array with increased numbers of clones, particularly in the telomeres of each chromosome, is available for use in carriers of structural chromosome abnormalities to detect, for example, translocation chromosome imbalance involving duplication and deletion of often small segments of these chromosomes. Alternatively, for whole-chromosome copy number analysis only, it is also possible to use arrays of chromosome-specific PCR libraries (15), but these arrays have not yet been extensively validated for PGS.

Array CGH was the first technology to be widely available for reliable, accurate, and relatively fast 24-chromosome copy number analysis and is now used extensively around the world despite the relatively high cost of testing multiple samples (Table 2). The first pregnancies and live births following PGS using array CGH to analyze copy number in the first polar body were reported in 2010 (16, 17). Subsequently, the European Society for Human Reproduction and Embryology (ESHRE) PGS Task Force organized a pilot study testing both first and second polar bodies for advanced maternal age and reported a high incidence of copy number abnormalities and a high concordance for predicted maternal aneuploidies in the corresponding zygote (18, 19). Detailed analysis of those pilot study data revealed a high incidence of multiple meiotic errors in individual oocytes, predominantly caused by premature predivision of sister chromatids (20). Because single sister chromatids segregating to the metaphase II oocyte then segregate randomly, about one-half of all chromatid errors in the first meiotic division were balanced in the second division with the chromatid segregating to either the second polar body or the zygote. Thus, screening only the first polar body does not accurately predict the aneuploidy status of the corresponding zygote.

More recently, separate biopsy of the first and second polar bodies and array CGH analysis was followed by analysis

TABLE 2

Comparison of available technologies for 24-chromosome copy number analysis.

Method	Duration of test	Complexity	Equipment cost	Reagent cost	Resolution	Pros and Cons
CGH	12–72 h	Medium	Medium	Low	Low	Low cost Skilled Labor intensive
Array CGH	12–24 h	Medium	Medium	Medium	Medium	Robust Scalable
Digital PCR	8 h	Medium	Medium	Low	Low	Low cost Scalable Rapid Polar body analysis only
Real-time quantitative PCR	4 h	Medium	Medium	Low	Low	Low cost Not scalable without additional equipment Multiple cell samples only
SNP microarray	16–72 h	High	High	Medium	High	Genome-wide analysis Quantitative and marker analysis Parental origin
Next-generation sequencing	15 h	High	High	Medium	Low	Scalable with multiplexing

Note: CGH = comparative genomic hybridization; PCR = polymerase chain reaction; SNP = single-nucleotide polymorphism.

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of the whole embryo at cleavage stages on day 3 after intracytoplasmic sperm microinjection in women of advanced maternal age (21). This demonstrated that 93% of the aneuploidies detected in a small series of cleavage-stage embryos were associated with copy number changes in the polar bodies and therefore of female meiotic origin. However, as in another study (22), there were false positive predictions, some of which appeared to be due to amplification bias, particularly in first polar bodies, and some of which may demonstrate biologic processes such as chromosome lagging.

Several RCTs of the use of array CGH for advanced maternal age and other indications are ongoing. The first RCT to be reported involved young good-prognosis patients who elected to have a single blastocyst transfer to avoid the complications of multiple pregnancy (23). That study compared implantation and ongoing clinical pregnancy rates with single blastocysts selected on the basis of embryo morphology alone versus single euploid blastocysts of good morphology and demonstrated a significant improvement in implantation and ongoing pregnancy rates with array CGH (42% vs. 69%, respectively, per single blastocyst transfer). In another retrospective study, cleavage-stage biopsy and array CGH were used in carriers of reciprocal or Robertsonian translocations to detect both translocation chromosome imbalance and aneuploidy and reported higher pregnancy and live birth rates than previously reported for FISH-based testing of the translocation chromosomes alone (24). Although only 61% of cycles had an embryo transfer, clinical pregnancy rate was 71% per transfer with an implantation rate per embryo transferred of 64%.

DIGITAL PCR

A novel approach to 24-chromosome copy number analysis in polar bodies is the use of digital PCR, which was developed for cancer studies (25). This is a method that counts the presence of chromosome-specific PCR target DNA by limiting dilution of the DNA after lysis of each polar body. Thus it avoids any need for whole-genome amplification and any associated amplification bias. The polar bodies are simply lysed and the lysate pipetted into eight separate wells. A multiplex PCR is then performed, followed by detection of chromosome-specific products in each of the wells. To control for amplification failure or allele dropout, multiple target sequences are ampilifed per chromosome. Under the right conditions, the number of wells positive for each chromosome-specific PCR product then reflects the number of DNA target molecules, i.e., chromatids in the polar body. This should normally be two in the first polar body and one in the second, predicting that the fourth chromatid has been segregated to the zygote. This approach is still being validated, but initial studies have confirmed that most copy number errors in the first meiotic division are caused by premature predivision of sister chromatids resulting in three copies or only one in the first polar body (Daser, personal communication). When coupled with robotics and high-throughput platforms for PCR, this technology is both rapid and low cost, offsetting the cost of analyzing two samples for each fertilized oocyte. The technique is intended only for polar body analysis and is particularly relevant in countries such as Germany in which legal restrictions prevent PGS on embryos beyond the pronucleate stage of development. Digital PCR could presumably be used with single blastomeres biopsied from cleavage-stage embryos, but analysis of cells in S phase may result in errors.

SNP ARRAY

A single-nucleotide polymorphism (SNP) is a DNA sequence variant in which, at a particular position or locus, one of two or more nucleotides may be present on different chromosomes within a population. To date, almost 40 million SNPs

have been validated, spread across the genome but mostly in noncoding regions. Biallelic SNPs, in which one of two bases is present, referred to generically as A and B, are valuable markers, and hundreds of thousands of SNPs can be genotyped simultaneously with the use of SNP arrays. Furthermore, for molecular cytogenetics, analysis of the ratio of the intensity of the B to the A alleles at heterozygous loci allows the detection of duplications and deletions from whole chromosomes to small regions with high resolution. Normally, where both chromosomes are present, there should be three bands representing AA, AB, and BB loci at a ratio of 0, 0.5 and 1. In the duplications, the B-allele ratio at heterozygous loci splits into two bands representing loci that are either AAB or ABB. In deletions, loss of heterozygosity (LOH) is detected by the absence of the heterozygous band. SNP arrays also have the advantage that the parental origin of any abnormalities can be investigated by genotyping the parents, allowing the detection of, for example, uniparental disomy.

The use of SNP arrays for chromosome copy number analysis and PGS has been pioneered by several groups, each of which used different approaches. Kearns et al. optimized their lysis and MDA protocol for whole-genome amplification from single and small numbers of cells to reduce amplification bias (26). This then allows conventional SNP copy number analysis simply by examining each chromosome for abnormalities in the B-allele ratio and for LOH. Treff et al., in contrast, used statistical methods to examine the intensity and assign a copy number at each SNP locus across the chromosome (27). The copy number assignment for the whole chromosome is then based on the copy number of the majority of loci. By applying a quality threshold and excluding two results, the accuracy of this approach was reported to be 98.6% for the 72 cells analyzed. Furthermore, a prospective blinded nonselection study of embryo biopsy and retrospective copy number analysis demonstrated a high predictive value for implantation or pregnancy failure associated with the transfer of aneuploid embryos (28). Copy number analysis of trophectoderm samples with the use of this method, combined with vitrification of the biopsied blastocysts and thawing and transfer of euploid blastocysts in a later cycle, resulted in high implantation and live birth rates: 73% per embryo transfer with an implantation rate of 65% per blastocyst transferred (29). It was also used successfully to detect translocation chromosome imbalance but only at moderate resolution down to ~10 Mb (30). Finally, Rabinowitz et al. developed a bioinformatics algorithm using parental SNP genotypes to improve the accuracy of genotyping of single cells and used this and a number of other proprietary algorithms to analyze chromosome copy number in blastomeres from cleavage-stage embryos (31).

An alternative approach is to use mendelian analysis of the SNP genotypes of the parents and single blastomeres or trophectoderm cells biopsied from each embryo to identify four sets of informative SNP loci across each chromosome that represent the four parental chromosomes, and then to generate a karyomap of the embryo showing the parental origin of each chromosome or chromosome segment (32). This requires the phasing of the A and B alleles at heterozygous loci in each parent, which for chromosome copy number analysis, can be achieved with the use of an embryo as a reference, because the embryo would normally only inherit one chromosome from each parent. With the use of this approach, trisomies of meiotic origin, in which two chromosomes with different patterns of recombination are present in the embryo, can be identified by the presence of both chromosomes from one parent in overlapping segments of the chromosome. Conversely, monosomies or deletions can be identified with high resolution simply by the absence of either chromosome from one parent. Thus, karyomapping is able to identify copy number abnormalities exclusively based on the genotype of the embryo and completely avoids the problems associated with quantification after whole-genome amplification. Of course, duplications of whole chromosomes or chromosome segments that are sequence identical can not be identified. For single-blastomere analysis this could be an advantage, because mitotic duplication of chromosomes resulting in chromosome mosaicism during cleavage would not be detected. However, karyomapping does not exclude quantification, and the combination of the two approaches would provide a powerful method that would identify all types of chromosome abnormality and their parental origin.

REAL-TIME QUANTITATIVE PCR

Although Treff et al. (33) pioneered the use of SNP arrays for copy number analysis, the time, cost, and complexity of SNP analysis, particularly the need to vitrify biopsied blastocysts, are restrictive, although there is increasing evidence that it may improve implantation and live birth weights (34). An alternative method for 24-chromosome copy number analysis that uses real-time quantitative PCR (qPCR) was therefore developed and extensively validated (33). With this method, a preamplification step, followed by a high-order multiplex PCR reaction in a 384-multiwell plate format, is used to amplify at least two sequences on each arm of each chromosome. Real-time qPCR is then used for the rapid quantification of each product, allowing a comparison across the genome. To avoid amplification bias from whole-genome amplification, the multiplex PCR is performed on the sample directly to ensure accurate copy number analysis and therefore is only applicable to multiple-cell trophectoderm samples. However, biopsy and analysis can be completed in only 4 hours, facilitating the fresh transfer of single euploid blastocysts in the same cycle (35). The only limitation with the technology at present is the limited number of samples, currently two on each plate, which can be run on the available equipment. However, the use of loading robots and running the analysis overnight allows higher throughput but extends the time taken to analyze all of the samples.

NEXT-GENERATION SEQUENCING

The rapid development of next-generation sequencing (NGS) technologies since James Watson was the first person to have their genome sequenced and published on the internet in May 2007 is remarkable. Tens of thousands of individuals have now had their entire genome sequenced, and efforts have begun to understand all the variants from the reference sequence that personal genomics identifies (36). It was

therefore inevitable that attempts would be made to use NGS technologies for preimplantation genetics.

Various NGS technologies are available (37). Typically, however, NGS involves fragmenting the sample DNA into small 100–200-basepair fragments and ligating linker oligonucleotides to either end of each fragment, one of which can include a short sequence that effectively "bar codes" the DNA from that sample. Multiple samples can then be processed together in a single sequencing cell in which the DNA fragments hybridize via the linker oligonucleotide to complementary oligonucleotides bound to the surface of the sequencing cell. Hundreds of thousands of these fragments are sequenced in parallel by the successive addition and removal of fluorescent nucleotides and ultrahigh-resolution imaging. The sequence of each fragment is then compared with the reference genome using dedicated software to complete the sequence. This process is continued until a sufficient "read depth," i.e., sequencing of multiple fragments from the same genomic region, is acquired for accurate sequencing of the required proportion of the genome.

For PGD, Treff et al. used a targeted NGS strategy and a multiplex PCR reaction that included both the mutation site and the chromosome specific target sequences required for qPCR (38). This strategy reduced the read depth necessary for accurate sequencing of the mutation site, which reduces the time required and cost. In parallel, qPCR of the multiplex PCR products provided rapid analysis of chromosome copy number.

For chromosome copy number analysis by NGS, the principle is straightforward (37). The whole-genome amplification products from the embryo samples are simply fragmented and sequenced and the read depth within successive regions of each chromosome compared across the genome. Because the number of fragments from a particular chromosome should be proportional to the copy number, trisomy or monosomy will result in greater or less read depth, respectively. Using this approach with trophectoderm samples from a series of blastocysts, both whole-chromosome aneuploidy and translocation-chromosome imbalance has been demonstrated with an average read depth of only $\times 0.07$ and coverage of as little as 5% of the genome (39).

CONCLUSION

The choice of which of the available technologies reviewed here for 24-chromosome copy number analysis are selected by clinics depends on a multiplicity of factors (Table 2). These include, for example, preferences for biopsy method, fresh versus frozen transfer, the turnaround time of the test, and whether or not the clinic wishes to set up an in-house facility or outsource to a service lab. All of the technologies are highly accurate. However, particularly at the single-cell level, the requirement for whole-genome amplification makes them susceptible to amplification bias and cell-cycle artefacts. On the other hand, those techniques that use PCR to amplify from the samples directly, such as digital PCR and real-time qPCR, are restricted to use on polar bodies and multiple-cell trophectoderm samples, respectively. A priori, SNP arrays or NGS-based methods for copy number analysis are likely to be the most accurate and informative, because they use sequence data from thousands of loci across each chromosome (37). However, the methodologies involved are more complex and the cost of the equipment is high, so these tests are probably going to be available only from the larger service labs. NGS platforms are currently designed for high throughput and accurate sequencing of whole genomes or exomes for postnatal and cancer applications. It is likely to be some time, therefore, before protocols and equipment optimized for flexible low- to medium-throughput applications in preimplantation genetics are developed and widely adopted.

Another important factor is scalability. If the time, effort, and cost of a technique increase linearly with the number of samples to be processed, laboratories can be quickly overwhelmed and turnaround times compromised. Conventional CGH is relatively straightforward to set up in house and the reagent costs are low. However, the interpretation of the results is highly skilled, and processing large numbers of samples is time consuming. Array CGH, in contrast, has been widely adopted because it is a robust technology with a turnaround time as short as 12 hours. It is also scalable with decreased cost per sample as increased numbers of samples are processed together. In addition, the same platform can be used for detection of translocation-chromosome imbalance and with dedicated prenatal and cancer microarrays. Real-time qPCR has been extensively validated, it has the fastest turnaround at 4 hours, allowing fresh transfer of blastocysts, and the cost of the reagents is relatively low. However, the equipment used currently allows processing of only very small numbers of samples and is therefore less scalable, because it requires multiple platforms.

At present, there is ongoing debate about the optimum time of biopsy for chromosome copy number analysis (22, 39–43). Clearly, the largest reported increases in implantation and live birth rates to date have been with blastocyst biopsy, which is to be expected because there has been a double selection for normally developing euploid blastocysts. Blastocyst biopsy is therefore a good choice for good-prognosis patients and particularly for those wishing to have elective single-embryo transfer to avoid the complications associated with multiple pregnancy (23). However, the cleavage-stage embryos of some poor-prognosis patients may implant and develop in utero but not develop to the blastocyst stage in vitro. From this point of view, polar body analysis is applicable to all patients and fertilized embryos and focuses exclusively on female meiotic errors, which are known to be the predominant cause of pregnancy loss, abnormal pregnancy, miscarriage and affected live births. This approach is, by definition, a screening test in the strict sense, because it can not provide any information about paternal meiotic errors or chromosome abnormalities arising after fertilization. A lower diagnostic accuracy may be tolerated as long as there is an overall improvement in healthy live birth rates. ESHRE has organized a large multicenter RCT of polar body analysis, which is scheduled to be completed in the next 1–2 years.

Polar body analysis also provides important prognostic information for couples about the origin of aneuploidies, the likelihood of pregnancy using their own eggs, and

whether they should consider egg donation as an alternative. In this respect, the use of SNP genotyping of the couple and their embryos to identify meiotic trisomies and monosomies by karyomapping has the advantage that meiotic errors from either parent can be identified even in single cleavage-stage blastomeres, providing enhanced prognostic information, for example, in cases where there may be an increased risk of aneuploidy from both partners. Thus, cleavage-stage biopsy, which is well established and remains widely practiced, in combination with karyomapping may be another effective strategy. Particularly if it can be combined with quantitation, karyomapping should also allow the detection of uniparental disomy that has been detected at the blastocyst stage (22).

It is clear from this review of available technologies that we are some way off from having a small benchtop box in the IVF lab into which samples can be placed for rapid, accurate, and low-cost 24-chromosome copy number analysis. However, all of the studies involved in developing the various methods have reinforced the reality that chromosome aneuploidy is common in embryos following IVF, even in younger women, and is a major factor in IVF failure. Furthermore, we continue to learn more about their origins and evolution in preimplantation development. It seems unlikely that we will ever screen all embryos, particularly if it requires invasive and time consuming biopsy procedures. However, for high-risk patients testing for 24-chromosome copy number is becoming increasingly established as an integral part of best clinical practice.

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