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