Embryo selection in IVF: is polar body array comparative genomic hybridization accurate enough?

Paul N. Scriven1,2,* , Caroline Mackie Ogilvie2,3, and Yacoub Khalaf2,4

1Division of Genetics and Molecular Medicine, King’s College London, School of Medicine at Guy’s, King’s College and St Thomas’ Hospitals, London SE1 9RT, UK 2Guy’s and St Thomas’ Centre for PGD, Guy’s and St. Thomas’ Hospital NHS Foundation Trust, London, UK
3Cytogenetics Department, Guy’s and St Thomas’ NHS Foundation Trust, 5th Floor Tower Wing, Great Maze Pond, London SE1 9RT, UK
4Assisted Conception Unit, Guy’s and St. Thomas’ Hospital NHS Foundation Trust, London, UK

*Correspondence address. E-mail: paul.scriven@kcl.ac.uk
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ABSTRACT: The emergence of the array comparative genomic hybridization technique (aCGH) is considered an advance in preimplantation genetic testing. Analysis of the recently published pilot study using polar body aCGH indicates that the test accuracy compares favourably with the fluorescence in situ hybridization technique although a substantial number of euploid zygotes are still likely to be excluded incorrectly. A sound argument against selection in principle has recently been published, based on accumulating evidence that potentially all embryos can now be cryopreserved and transferred in subsequent frozen replacement cycles without impairing pregnancy rates. We suggest that vitrification and serial transfer without testing are likely to give patients the best chance for a successful pregnancy, and avoid the use of an expensive technology.

Key words: PGS / PGD / aCGH / diagnostic accuracy

Preimplantation genetic screening (PGS) for aneuploidy, using chromosome-specific fluorescence in situ hybridization (FISH) probes, has been offered extensively at many centres, with the aim of selecting euploid embryos for transfer and thus improving the efficiency of assisted conception. Randomized controlled trials of PGS using FISH have failed to demonstrate a beneficial effect on the live birth rate (Mastenbroek et al., 2011), and the advent of new technology has led to the ESHRE-funded pilot study on PGS using the recently developed array comparative genomic hybridization (aCGH) technique to test polar bodies and hence to predict the chromosome status of the corresponding zygote (Geraedts et al., 2011). This pilot study has concluded that the ploidy of the zygote can be predicted with an acceptable accuracy using the new technology. We believe that this is worthy of further analysis. We have used the study results to estimate the measures of diagnostic accuracy and applied a theoretical model developed for preimplantation genetic testing for further sensitivity analysis and to provide a theoretical comparison using 23-chromosome FISH (Scriven and Bossuyt, 2010). In brief, the theoretical model calculates diagnostic accuracy measures uncomplicated by mosaicism for any number of chromosomes tested greater than two. The input values are the degree of aneuploidy per chromosome and the accuracy per chromosome, defined as the proportion of normal diploid nuclei with a normal signal pattern. In our analysis of the data from Geraedts et al. (2011), we excluded the (not insignificant) 31 of 226 (14%) zygotes where results were not obtained from both polar bodies. Of the remaining 195 zygotes, index results with unknown outcome were assigned in proportion to those with known outcome. Out of 195 zygotes, 55 had a normal index result and 140 had an abnormal index result. A total of 156 outcome results were obtained, of which 38 were normal and 118 were abnormal. There were eight discrepant outcome results: seven incorrect abnormal index results and one abnormal index result with a different abnormal outcome. The 156 zygotes therefore comprised 31 (38–7) with normal index results and 125 (118+7) with abnormal index results. The remaining 39 zygotes (195–156) did not have the outcome results: of which 24 had normal index results and 15 had abnormal index results. As there were no incorrect normal index results in the confirmed findings, all of these 24 unconfirmed normal index results were assigned a normal outcome. There were 118 of 125 (0.944) correct confirmed abnormal index results; 14 of 15 (15 × 0.944) of the unconfirmed abnormal index results were therefore assigned an abnormal outcome and 1 of 15 (15 × 0.056) a normal outcome. Following the assignment of all 195 index results, there were 8 (7+1) false abnormal results, 132 (140–8) true abnormal results, no false normal results and 55 true normal results. In summary, therefore, of the 195 zygotes, 63 (55+8) were calculated to have a normal outcome and 132 (132+0) have an abnormal outcome. Table I shows the outcome
measure calculations and their precision. The theoretical model (Scriven and Bossuyt, 2010) converged on the empirical study accuracy when the abnormality rate per chromosome was 4.8% (equating to the 67.7% abnormality prevalence in the pilot study estimated from all 195 zygote index results) and the accuracy for normal copy number was 99.4% per chromosome tested. We conducted the sensitivity analysis using this accuracy measure applied to the theoretical model to investigate the effect of the aneuploidy rate on the predictive value of the test (analogous to testing patient groups that might have a different degree of aneuploidy, for example, younger or older women).

Table I shows that the accuracy using the aCGH test was estimated to be 95.9% with 100% sensitivity and 87.3% specificity. With a chromosome abnormality prevalence of 67.7%, the predictive value was estimated to be 94.3% for an abnormal test result and 100% for a normal test result. False abnormal test results were estimated to be 4.1% of the total test results and 12.7% of the euploid zygotes. The comparative FISH illustration estimated the predictive value to be 80.4% for an abnormal test result and 93.4% for a normal test result, with 16.2% of the total test results and 50.4% of the euploid zygotes incorrectly diagnosed to be abnormal. Figure 1 shows that, using the aCGH theoretical model, the predictive value of a normal test result approached 100% across the aneuploidy range and that the predictive value of an abnormal test result declined rapidly at lower levels of aneuploidy: 90% with 53% prevalence (3.2% per chromosome); 50% (equivalent to tossing a coin) with 11% prevalence (0.51% per chromosome). Figure 1 Sensitivity analysis of the predictive value, varying the aneuploidy rate (18% per chromosome equates to a 99% chance of aneuploidy for at least 1 chromosome testing 23 chromosomes). Npv, negative predictive value; ppv, positive predictive value.

We conclude from our analysis (and agree with Geraedts et al.) that the accuracy of the polar body array comparative hybridization test is likely to be high [at least for patient groups where the overall prevalence of aneuploidy is at least around 50% (i.e. ~3% per chromosome)], and compares favourably with what might be expected using 23-chromosome FISH. However, the predictive value of an abnormal aCGH test result is likely to be significantly <100% and a substantial proportion (>10%) of normal zygotes may therefore be excluded incorrectly. In our opinion, whether the accuracy achievable using polar body array comparative hybridization for PGS is good enough to improve the patient outcome remains debatable and we look forward to seeing the result of the planned randomized trial.

Pregnancies with chromosome aneuploidy are a natural occurrence for couples who do not need assisted conception. A test which identifies aneuploidy with 100% accuracy would indeed be a huge leap forward in the struggle to improve pregnancy success rates. (Although even the transfer of a euploid embryo does not, of course, guarantee a live birth and a healthy child.) However, such a test has not yet been developed, and in our view, centres should consider carefully their use of inaccurate tests which result in the destruction of healthy embryos.

However, regardless of the effectiveness of aneuploidy screening in delivering acceptable live birth rates over a shorter time period, the application of an expensive technology for embryo selection to improve IVF success rates may be unnecessary with the development of highly effective and efficient cryopreservation protocols such as...
vitrification, as pointed out by Mastenbroek et al. (2011b). These authors argue the case against embryo selection in principle, based on accumulating evidence that potentially all embryos can now be cryopreserved and transferred in subsequent frozen replacement cycles without impairing (and possibly improving) pregnancy rates compared with when selection is used. We agree that in such a scenario the use of a selection method cannot increase (but could decrease) the live birth rate per stimulated IVF cycle when all embryos are serially transferred (albeit potentially reducing the time to first delivery).

Indeed, there may be an argument for vitrification of all embryos without fresh transfers, considering potentially superior perinatal outcome of births resulting from transfer of thawed blastocysts (Wikland et al., 2010). In that study, more singletons born after transfer of fresh blastocysts were small for gestational age compared with singletons born after transfer of vitrified blastocysts.

In conclusion, given the inherent suboptimal accuracy of any testing, we agree with Mastenbroek et al. that the time has come to re-assess conventional thinking on embryo selection in the light of recent developments, and stress the importance of well-designed studies before routine clinical use and of the need to avoid the ‘technological imperative’ in our approach to assisted reproduction.

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P.N.S., C.M.O. and Y.K. conceived and critically reviewed the manuscript. P.N.S. performed the calculations and drafted the manuscript.

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Conflict of interest
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References