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Editorial Open Access

A new era of PGS for IVF – will it yield the anticipated improved efficiency?

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At the outset it is necessary to establish the acronyms that geographically have a different meaning. In the UK and elsewhere it is customary to separate out the screening of chromosomes (Preimplantation Genetic Screening – PGS) from diagnosing a single gene disorder - Preimplantation Genetic Diagnosis (PGD). In some regions, notably the US, both processes - PGS and PGD - are often included in the over-arching term, PGD. This short article deals only with PGS (perhaps better described as Chromosome Screening). However, it should be noted that with some platforms it is becoming increasingly easier to deliver information on not only the full complement of chromosome information, including a translocation, but also the diagnosis of a monogenic diseased state as well as HLA matching, from a single cell [1] – an exciting prospect indeed!

Clinical assessment over many decades has established that aneuploidy is the main cause of early miscarriage and congenital birth defects, and is the most common chromosome abnormality in humans. The majority arise from errors in maternal meiosis1 [2]. This fact on its own was important for considering how to improve outcome during an IVF cycle, but it was also believed that many of the failures of implantation were due to aneuploid embryos. Hence, more than two decades ago solutions to assessing aneuploidy post insemination resulted in the biopsy of a cell (or two) from a day 3 preimplantation embryo and examining the chromosomes by fluorescent in situ hybridisation (FISH) – a fast relatively low-cost technology for assessing first 5, then up to 9 chromosomes. After years of utilisation of FISH for assessing ploidy in IVF embryos, it became apparent that the technology was not producing the desired results, and indeed could be deleterious [3]. This was due primarily to three major contributing factors; 1) the FISH technology itself was flawed and open to major interpretational errors; 2) only a fraction of the whole chromosome complement was being examined; and 3) it was believed that the removal of a single blastomere on day 3 was, in a significant number of cases, not representative of the embryo due to mosaicism. Hence FISH resulted in very high levels of false positive and false negative data. Whether the level of mosaicism was a result of the FISH technology per se, or a biological axiom was unclear. What became obvious was that FISH technology for IVF was the key problem; the true incidence of mosaicism, with reliable technology, is still to be fully ascertained.

However, the clinical data demonstrating high aneuploidy rates in pregnancies resulting in miscarriage still remained. Further work using a more reliable technology, such as metaphase comparative genomic hybridisation (mCGH), which was unsuitable for 'in-cycle' IVF and ET ('fresh' ET), provided strong evidence that aneuploidy was indeed high in the eggs and embryos of all patients, and a particular problem in women with advancing reproductive age. Therefore, the rationale still prevailed – that for women of advancing age, those with recurrent implantation failure or early miscarriage – the need to try to identify aneuploid embryos was essential. The challenge was how to do this! What was clear was the strategy first needed a robust technology that assessed all the 24 chromosomes, that analysis could be undertaken rapidly enough to ensure ET without the need to freeze all embryos, and that the cell(s) being evaluated would be informative of the embryo.

In 2008 Bluegnome (Cambridge, UK) entered the arena with ground-breaking technology, '24sure' array CGH (aCGH), which today offers highly accurate 24 chromosome assessment from single cells within a 12 hour period. This was set to revolutionize what we could do, and what we could learn. The next challenge at that time was from where the information would best be provided? Mosaicism at day 3 remained a concern and there was no data on the ploidy concordance between inner cell mass (ICM) and trophoblast to encourage the use of only trophoblast cells from the blastocyst; furthermore, the freezing of the latter would have been mandatory. At that time the success of blastocyst freezing was not as efficient as it is now. Working with Bluegnome in 2008 the CARE Fertility Group decided the best option at that time was the polar body. The use of polar body 2 (PB^2) along with PB^1 was a prospect, but it meant twice the number of tests, twice the cost, and the data from PB^2 was, at that stage of development, exceptionally noisy and much less clear to interpret. Recognising that paternal meiotic and cleavage mitotic errors could still be introduced post PB removal, it was, nevertheless, believed that the vast majority of the errors seen in embryos resulted from meiosis 1 and would therefore be detected in the PB. It was assumed that an aneuploid oocyte derived only aneuploid embryos. Hence, by examining PB^1 and detecting aneuploid oocytes we would at least be eradicating a high percentage of aneuploid embryos – deriving a better prospect for increasing the efficiency of implantation and birth than any currently subjective microscopic assessments. Moreover, not all patients would take the risk of a few zygotes developing to blastocyst, so by using PB analysis a Day 3 transfer could take place.

The first use of this technology to a difficult case resulted in a birth in 2009 [4]. This was followed shortly by a statement from the European Society for Human Reproduction (ESHRE) consortium denouncing the use of FISH for aneuploidy assessment but supporting the use of aCGH for PB testing. Subsequently, ESHRE undertook their own proof of principle trial using both PB^1 and PB^2 and their recent publication evidences a very high correlation to zygote aneuploidy [5]. However, there is still a need for a large randomised clinical trial (RCT) to prove the science can affect clinical outcome, giving sustainable support for the use of PB chromosome screening. But such RCT's are notoriously difficult to fund and achieve patient compliance. The case for the use of both PBs in routine IVF depends as much on economics as biology. Indeed, the ESHRE study doesn't provide enough biological information to say whether PB^2 is more representative of aneuploidy than PB1 and hence better for predicting outcome or that both PB^1+PB^2 are required. ESHRE is planning its own RCT – it is hoped its outcome can answer these questions. However, from current studies

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detailed biological information is illuminating our understanding of the incidence and frequency of individual aneusomies in oocytes and embryos, and, for example, that C:G content and chromosome length correlate to the probability of segregation error.

Meanwhile, to date, several platforms for assessing full chromosome complement of cells have arisen. With each technology care is needed in assessing and comparing data across platforms – in any direct comparison there are traps! (Table 1). In previous comparative

Technology	Advantages	Disadvantages	Comments
FISH	• Very low cost per test • Fast • Low cost capital equipment	• Only 7-9 chromosome probes • Each probe is only 1 loci per chromosome and hence may miss arm/segmental changes • Analysis subjective due requiring to operator interpretation	• Largely discontinued after a number of randomized clinical trials showed limited or no efficacy1.
Copy number array comparative genome hybridisation (aCGH)	• Low cost per test • Results in under 12hrs • Easy to interpret and non-subjective • Robust results – hundreds of results per chromosome • Resolution to <2.5MB2 • Detects whole chromosome changes, arm changes, segmental imbalances, 69 XXY triploid, mosaic aneuploidies (TE biopsy)	• Cannot determine parental origin of aneuploidy • Cannot determine copy neutral events (loss of heterzygosity, uniparental disomy, 69 XXX triploidy)	• Suitable for the detection of chromosomal imbalances from polar body 1/2, blastomere, trophoectoderm biopsy, all without vitrification. • Also can be used for detection of imbalances from reciprocal/robertsonian translocation and inversion carriers
Single nucleotide polymorphism (SNP) arrays for copy number imbalance analysis.	• Robust results – thousands of results per chromosome • Determines parental origin of aneuploidy when used with parental genotype data. • Detect copy neutral events plus whole chromosome changes, arm changes, segmental imbalances, triploidy (not tetraplpoidy)	• High cost per test • No results in under 24hrs • Results require complex algorithms and expert knowledge to analyse results • High capital equipment start-up costs • Karyomapping and Parental Support (GSN) require parental DNA analysis in advance to haplotype embryo results due to low initial SNP calling	• Cannot detect chromosomal imbalances from TE biopsy without vitrification. • Can be possibly be used for monogenic PGD diagnosis using parental genotype data • Also can be used for detection of imbalances from reciprocal/robertsonian translocation and inversion carriers
Real time PCR SNP analysis for copy number imbalance analysis	• Low cost per test • Fast – less than 6hrs	• Very high capital equipment start-up costs • Results require complex algorithms and expert knowledge to analyse results • Only detects whole chromosome changes , cannot detect chromosome arm changes or segmental imbalances	• Use initially has been for the analysis of TE biopsy within fresh cycle transfer. • Full details not yet published

Table 1: Pros and cons of different forms of preimplantation genetic screening analysis technology.

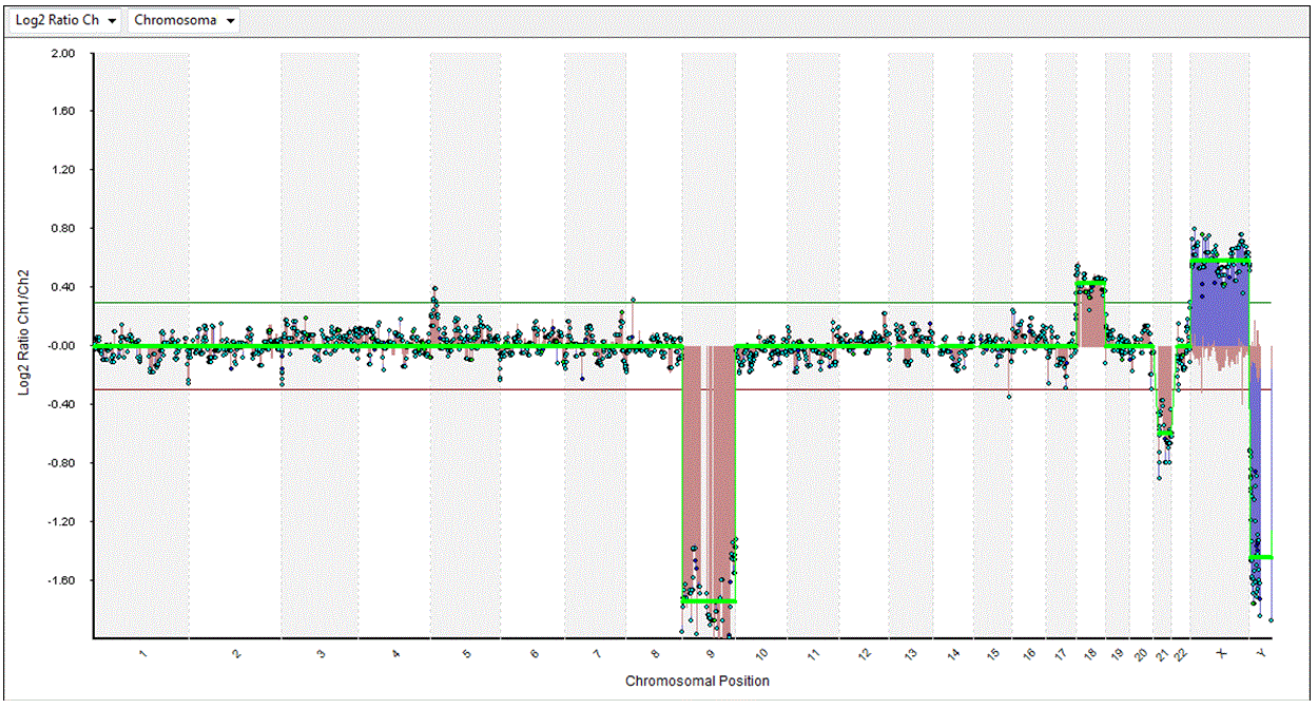

Figure 1: PB[1] Chromatid 18 gain and Chromatid 21 loss and Chromosome 9 loss (run against male control ? hence gain ch X and loss Y).

commentaries, for example, those who have contrasted mCGH to aCGH needed to understand that there exists inherently more signal noise in the former giving an erroneous assessment of chromosome gains, in particular. Furthermore, new evidence from PB testing demonstrates that premature sister chromatid separation is prevalent, changing the dogma and role of non-disjunction in aneuploidy, and requiring the emerging technologies to take this into account when evaluating meiotic error (Figure 1). Gabriel et al. [6] revealed that single chromatid errors (SCE) were over 11 times more common than whole chromosome errors!

There are also decisions to be made about which cell stage to assess. Clearly, if mosaicism at the cleavage stage proves to be acceptably low then day 3 is more advantageous than PB as it incorporates paternal, and, up to that point, cleavage-induced aneusomies. For patients for whom blastocyst assessment is appropriate, emerging data implies a very high concordance between ICM and trophoblast [7].

Even if embryo assessment proves the most efficient, there may still remain a place for PB testing for those patients and jurisdictions that consider embryo biopsy inappropriate (for ethical or regulatory reasons). The future, however, is likely to witness a significant number of patients avoiding fresh embryo transfer altogether. The very high success rates of blastocyst vitrification will permit trophoblast testing before freezing to prevent cryopreservation of aneuploid embryos, and thawing and transfer of single euploid blastocysts to minimise the risks of two major enemies of IVF – multiple pregnancy and ovarian hyperstimulation syndrome.

Aneuploidy assessment today has reached an exciting stage with several reliable high-technology solutions available to evaluate all chromosomes in either polar body, single cells of preimplantation embryos or the trophoblast. Will this new era of PGS screening give data that has biological relevance to spawn greater clinical efficiency? Or will biology yet have some interesting surprises, such as the correction of aneuploidy, or the selection of only (or enough) euploid cells for the ICM, for example? Promising new studies indicate that we are on the cusp of change – but we must never forget the lessons of the first PGS era!

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