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COMMENTARY

Embryo aneuploidy and the role of morphological and genetic screening

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Abstract Chromosome abnormalities are common among human oocytes and are usually lethal to any embryos they produce. It therefore seems logical that a reliable technique for distinguishing between normal and aneuploid embryos would be a useful tool for physicians and embryologists, assisting the choice of which embryo(s) to prioritize for uterine transfer. This concept has led to the development of a variety of methods for the detection of chromosome abnormalities in oocytes and embryos, most often referred to as preimplantation genetic screening (PGS). However, several well-controlled studies have been unable to show an advantage of chromosome screening in terms of pregnancy and birth rates. Some investigators have suggested that damage to embryos, sustained during cleavage-stage biopsy, might explain why PGS has not always provided the anticipated benefits. This paper asks whether there is evidence that a non-invasive, morphological analysis could allow chromosomally normal embryos to be accurately identified and reviews data from the most recent publication concerning IVF outcome following PGS.

KEYWORDS: aneuploidy, chromosome, embryo, morphology, preimplantation genetic diagnosis, preimplantation genetic screening

Introduction: chromosome abnormalities, embryos and screening

Data from numerous studies using a wide variety of techniques have demonstrated beyond doubt that a high proportion of human oocytes are affected by chromosome abnormalities. The phenomenon is closely associated with female age. For a woman over 40 years old undergoing IVF treatment, it is typical for more than half of the oocytes retrieved to be chromosomally abnormal (Fragouli et al., 2006, 2010a; Kuliev et al., 2003; Pellestor et al., 2003; Sandalinas et al., 2002). Embryos produced from chromosomally abnormal gametes display aneuploidy in all of their cells. Although such embryos occasionally produce children affected by chromosome abnormalities (e.g. Down syndrome), the vast majority either fail to implant or culminate in a miscarriage.

The high frequency of aneuploid embryos and the negative outcomes associated with their transfer during IVF cycles have led to the suggestion that efforts should be made to identify chromosomally normal embryos and ensure they are given priority for transfer to the uterus (Colls et al. 2007; Gianaroli et al. 2004; Munné et al., 1993). In theory, this should increase the likelihood that the embryos are viable, leading to improved implantation, pregnancy and birth rates and reduced incidence of miscarriage and Down syndrome. To date, most efforts to screen IVF embryos for aneuploidy have involved blastomere biopsy 3 days after fertilization of the oocyte, followed by screening of a limited set of chromosomes using fluorescent in-situ hybridization (FISH). However, clinical studies using this approach have yielded conflicting results in terms of outcome and several randomized trials have failed to demonstrate the expected benefits of chromosome screening using blastomere biopsy and FISH (Hardarson et al., 2008; Mastenbroek et al., 2007; Staessen et al., 2004).

It has been suggested that variability in the results of chromosome screening may be a consequence of technical

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problems (Munné et al., 2009, in press). Certainly, the reported accuracy of aneuploidy screening varies greatly between different laboratories (Baart et al., 2004; Coulam et al., 2007; Colls et al., 2007; Magli et al., 2007). Furthermore, embryo biopsy is likely to represent a cost to the embryo. If the biopsy is performed well, the cost may be small and easily offset by the improved identification of viable embryos, but if the biopsy is performed poorly or the embryos have too few cells at the time of biopsy, the implantation rate may be dramatically reduced.

This issue sees publication of a paper by Finn and colleagues (in press). This paper asks two important questions. Firstly, does embryo morphology hold any clues concerning the chromosomal status of an embryo? This is a highly attractive possibility, as a chromosome assessment based upon morphological analysis would allow embryo biopsy to be avoided altogether, resulting in an inexpensive test with little if any impact on the embryo. Second, they asked whether blastomere biopsy followed by FISH improved outcomes in their clinic. Given the current controversy surrounding the concept of embryo chromosome screening, any new data in this area is of great interest.

Biological perspectives

A number of previous studies have looked at the relationship between morphology and aneuploidy. Most investigations have confirmed that a link exists, but have found that correlations are weak and likely to be of little assistance in the selection of euploid embryos for transfer (Alfarawati et al., 2010; Eaton et al., 2009; Hardarson et al., 2003; Magli et al., 2007; Moayeri et al., 2008; Munné et al., 2007; Staessen and Van Steirteghem, 1998). To date, most studies have focused on embryos at the cleavage stage. This makes sense from the morphological perspective, as this is the phase when most visual assessments are routinely carried out and scoring systems are well developed. However, analysis at this stage is less appropriate from a biological standpoint, since the embryo genome is inactive until the 4–8-cell stage (Braude et al., 1988). It is likely that meiotic aneuploidies only begin to exert a negative effect on development and morphology once the embryo begins to rely upon its own genes around day 2 or 3.

This notion is supported by the data of Finn et al., which shows an identical incidence of aneuploidy among zygotes regardless of morphological grade (Z1/Z2 = 75% aneuploid; Z3/Z4 = 76% aneuploid). While chromosome imbalance appears to be invisible at the zygote stage, its influence increases as embryos progress through preimplantation development. A recent study, employing a more detailed chromosomal analysis of embryos at the blastocyst stage (day 5), reported a statistically significant link between aneuploidy, slower rate of development and poorer inner cell mass and trophectoderm grades (Alfarawati et al., 2010). One-half of the highest quality, grade-5 or -6, blastocysts were aneuploid, compared with 62.5% of embryos graded 1 or 2. Interestingly, morphologically poor blastocysts had a higher incidence of monosomy, abnormalities affecting several chromosomes and imbalance of large chromosomes, suggesting that these 'severe' forms of aneuploidy begin to impact development and morphology earlier than 'milder' types, such as trisomy (Alfarawati et al., 2010). This is in keeping with the observation that trisomies often survive into the first trimester of pregnancy, and sometimes beyond, while monosomies and anomalies affecting more than one chromosome or large chromosomes are extremely rare in clinical pregnancies.

Given the lethality of most forms of aneuploidy, it seems reasonable to expect that chromosome abnormalities will have a growing impact as time progresses, but what about the apparent association of aneuploidy and cleavage stage morphology reported by Finn et al. and others? The embryonic genome is only just becoming active at the cleavage stage, so is this not too early for meiotic chromosome imbalance to have a visible effect? A possible explanation is that the specific features that are assessed, in the case of Finn et al. multinucleation and symmetry, do not depend on disruption of gene expression resulting from aneuploidy. Anaphase lag, a common mechanism leading to loss of chromosomes, is often associated with formation of micronuclei. This may explain the apparent increase in multinucleation observed in chromosomally abnormal embryos. Additionally, errors of cytokinesis can lead to inappropriate division of chromosomes between daughter cells, potentially explaining the increased aneuploidy in embryos with uneven-sized blastomeres. Thus, multinucleation and evenness of cell size may provide an indication of mitotic errors (i.e. defects in mitosis, post-fertilization), but may be less useful for revealing meiotic errors. If this hypothesis is correct, an excess of chromosome losses (e.g. more monosomies than trisomies) would be expected in the embryos displaying multinucleation. This should be readily easy to confirm or refute.

Another possible explanation for association between aneuploidy and morphology prior to genome activation is that problems during early development, visualized as abnormal embryo morphology, are not related to aneuploidy itself, but are symptomatic of a more generalized oocyte deficiency, of which aneuploidy is just one of several symptoms. For example, oocytes with low or inappropriate levels of mRNA and proteins may be predisposed to producing morphologically abnormal embryos and also at high risk of chromosome malsegregation. Such oocytes may be especially vulnerable during the first couple of days following fertilization when the embryo is entirely dependent on resources supplied by the oocyte. Some data to support the concept that atypical levels of polyadenylated mRNA transcripts predispose oocytes to meiotic error has recently been presented (Fragouli et al., 2010b).

Clinical potential

Whatever the underlying biological cause, there does appear to be a link between aneuploidy and abnormal morphology at the cleavage and blastocyst stages. The clinical question is: can morphological analysis assist the identification of euploid embryos for uterine transfer? In the study by Finn et al., the probability of selecting a chromosomally normal embryo at random was 0.26. Whereas if transfer had been restricted to those embryos achieving the morphological appearance most closely associated with euploidy (Z1/Z2, even cell sizes, no multinucleated blastomeres),

the probability of selecting a euploid embryo would have been increased to 0.34. This modest improvement in the chance of choosing a chromosomally normal embryo echoes the findings of the recent study of aneuploidy and blastocyst morphology, which concluded that the aneuploidy rate observed on day 5 could be reduced from 56% to 48% if only embryos achieving the top two grades (5AA/6AA) were selected for transfer (Alfarawati et al., 2010). In reality, the reduction in aneuploidy is likely to be even lower, since inevitably many IVF cycles will not produce any embryos with the optimal morphological score on day 3 or day 5.

To summarize, it seems that the routine morphological analyses conducted at the cleavage or blastocyst stages do help to reduce the risk that a chromosomally abnormal embryo will be transferred. However, the improvements at both stages are relatively small and neither comes close to providing a definitive diagnosis of aneuploidy. Sequential embryo scoring, as utilized by Finn and colleagues, is a promising approach for maximizing the information gained from morphological evaluation, but in terms of avoiding aneuploidy it seems to be of relatively little value. The conclusion of the authors that zygote-screening-assisted selection of chromosomally normal embryos is not supported by the data presented. In fact, assessment of zygote morphology does not appear to add to any additional benefit beyond that obtained by analysis of multinucleation and cell size on day 2 or 3. At this time, the most reliable means of distinguishing normal from aneuploid embryos remain the invasive methods based upon embryo biopsy. Further investigations are required, but blastocyst biopsy, with analysis using either comparative genomic hybridization (CGH) or microarray-CGH, appears to represent the most accurate approach currently available (Schoolcraft et al., 2009).

The efficacy of preimplantation genetic screening

As well as reporting morphological data, Finn and colleagues have also provided a summary of their experience using preimplantation genetic screening (PGS), assessing the copy number of nine chromosomes using FISH in order to assist the transfer of euploid embryos in poor-prognosis patients. Although many laboratories have now moved to comprehensive chromosome analysis methods (e.g. CGH, microarray-CGH and single-nucelotide polymorphism array), a better understanding of the potential benefits and pitfalls of the previous generation of techniques remains desirable.

The conclusion of the study was that PGS had no affect on delivery rates for poor-prognosis patients with a good response to ovarian stimulation ($\geq$6 fertilized oocytes) and may have been detrimental to pregnancy rates for those with a low response (1–5 fertilized oocytes). However, there is some indication from the data that overall implantation rates might have been improved by PGS. This is an important point when considering single-embryo transfer, as increased implantation offers the possibility of maintaining pregnancy rates while reducing the number of embryos transferred. The proportion of transferred embryos that resulted in a pregnancy was 16% for PGS cycles compared with 12% for cycles without PGS. Unfortunately, the study was insufficiently powered to determine whether this apparent 33% increase in implantation was significant.

One of the main problems with the assessment of PGS performance in the study of Finn and colleagues was the heterogeneity and discordance between the PGS and control groups. The control group was composed of patients who originally intended to have PGS, but later decided against chromosome screening. Clearly, there are a variety of reasons why one patient might elect to go through with PGS, while another declines to proceed. The patients most committed to PGS are usually those with the most fraught IVF histories, who are often looking for answers and, in some cases, closure. In general, such patients would be expected to have worse outcomes than most others. The control group was not well matched for stimulation protocol, having a significant difference in the number of patients having agonist suppression versus antagonist cycles. Another difficulty is that 8% of the PGS cycles involved a frozen-embryo transfer, while all transfers in the control group were fresh. Given these problems, the assertion of the authors that 'the patient groups are well matched making this study comparable with randomized controlled trials' is not justified.

A disappointing pregnancy rate following PGS in the low-responder group was perhaps to be expected, since these patients had between one and five fertilized oocytes and presumably even fewer embryos actually suitable for biopsy. PGS is an embryo selection technique, designed to help identify the embryos with the highest probability of producing a viable pregnancy. Having the test does not increase an individual embryo's chances of implantation, indeed screening at the cleavage stage is likely to represent a small set-back to the embryo (Cohen et al., 2007). This may be more than compensated by improved embryo selection in instances where many embryos are available for transfer, as in such cases the probability of choosing the most viable by chance is reduced. However, in cases where there are few embryos, the selective advantage gained by screening is low and perhaps insufficient to offset the impact of biopsy. It is not clear how many embryos Finn et al. typically screened in cycles from low responders, but the number must have been considerably below five and may well have approached the number of embryos that would routinely be transferred to these patients in cycles without screening. Obviously, in cycles where the number of embryos available is equal to or less than the number typically transferred, PGS cannot offer any selective advantage and cannot be expected to improve pregnancy rates. Previous publications have suggested that, in the USA, PGS is not indicated for cycles with less than five embryos suitable for biopsy (Munné et al., 2003). In other countries, where it is typical to transfer fewer embryos, the threshold may be lower (e.g. screening in cycles with only three embryos would probably be appropriate for countries where single-embryo transfer is routine). Another issue is the number of cells biopsied. For PGS to be beneficial it is important that any impact on embryo viability is minimized. In the study of Finn et al., 7% of embryos underwent biopsy of two cells, a strategy shown to reduce implantation/pregnancy in both theoretical and clinical studies (Cohen et al., 2007; De Vos et al., 2009).

Given the limitations of the control group, the statistical power of the study and the low number of embryos available per cycle in the low-response group, it is hard to draw any firm conclusions from the study of Finn and colleagues concerning the efficacy of PGS. The data may be suggestive of an improvement in implantation rate, but not pregnancy rate. The results also appear to confirm previous observations that PGS offers little advantage and may even be detrimental in cycles with very low numbers of embryos. It will be interesting to see the outcome of future studies from this group and others, extending sequential morphological analysis to the blastocyst stage and utilizing the new comprehensive chromosome screening methods to assist embryo selection, but for the time being, detection of aneuploidy remains dependent on polar body or embryo biopsy.

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