specifically by polar body analysis. Furthermore, simply in principle, the incidence and extent of any chromosomal mosaicism can never be ascertained accurately from sampling small numbers of cells from preimplantation stage embryos. They also suggest that copy number analysis needs to be supplemented by molecular genetic analysis to detect, for example, ‘rescued’ trisomies which have eliminated one of the three chromosomes and can result in uniparental disomy (UPD) with serious consequences for later development. Again, we would argue that any of the possible outcomes of aneuploidy rescue, including diploid/aneuploid and UPD/aneuploid mosaicism, is potentially too serious clinically to risk detection by trophectoderm biopsy precisely because of the problem of mosaicism. This therefore is another strong argument for testing the chromosomal status of the oocyte at fertilization and we would draw a parallel with routine pronuclear screening to avoid the risk of triploidy resulting from dispermic fertilization or retention of the second polar body.

The optimal stage to biopsy embryos for aneuploidy testing will ultimately depend on the clinical indication, the patient’s age and the number and quality of embryos. For many women of advanced maternal age, the number and quality of embryos available will be too low to consider blastocyst biopsy. On the other hand, in young women of good prognosis, in which there may be a lower incidence of chromosomal mosaicism, blastocyst biopsy and testing by array CGH significantly improves ongoing clinical pregnancy rates following elective single blastocyst transfer (Yang et al., 2012).

Reply: Questions about the accuracy of polar body analysis for preimplantation genetic screening

Sir,

We thank Drs Fragouli and Wells and Drs Christopikou and Handyside for their interest in our paper (Capalbo et al., 2013). We welcome the opportunity to provide clarification and further stimulate discussion on this important topic.

We acknowledge the possibility that biological variation due to mosaicism may produce a low error rate during trophectoderm (TE) based prediction of inner cell mass (ICM) chromosome complement. However, this aspect is considered to have a very low impact on the evaluation of polar bodies (PB) chromosomal screening accuracy according to the existing literature, including the papers mentioned by Fragouli and Wells in their letter. A very high rate of consistency between the comprehensive chromosome screening (CCS) of different TE samples from the same blastocyst and between TE and ICM was reported when a single method for chromosome screening was applied (Johnson et al., 2010; Northrop et al., 2010). In the Johnson paper all CCS results obtained from different TE biopsies derived from the same blastocysts were concordant. Furthermore, ICM and their relative TE samples gave the same results in 96.1% of cases. In Northrop et al., considering only embryos producing interpretable results in at least three out of four embryonic sections, 46 of 47 (98%) embryos had at least three concordant results in the four embryonic sections analysed. It is also interesting to note that Fragouli herself (Fragouli et al., 2008) found 100% consistency during chromosomal screening of 10 ICMs and their relative TE samples by CGH analysis. The conclusion of the authors in their paper was that ‘data obtained from TE biopsies can generally be considered diagnostic of ICM aneuploidy’. In addition, we have also observed 97.1% consistency between ICM and TE from 70 good quality blastocysts reanalysed by 9-chromosome FISH (Capalbo et al., 2011b). The higher estimation of blastocyst TE chromosomal mosaicism observed by Fragouli et al. in 2011 (Fragouli et al., 2011) may have been biased due to the methodology of the study. In fact, the intrinsic differences in the performance of the three different chromosomal screening methods used in that study may have contributed to the accuracy of the assessment of biological mosaicism prevalence. Finally, the clinical predictive value of TE-based CCS was shown to be very high in a recent prospective double blind study (Scott et al., 2012). In addition, it is worth to remember that aneuploidies predicted by PB testing leads to constitutive aneuploidies in the embryo ruling out the mosaicism issue related to TE-based PB accuracy assessment. This is further supported by the results of our study where the analysis of one blastomere sampled on day 3 of embryo development was also included. Indeed, most PB false- and -negative assignments were based on two consecutive confirmation analyses at day 3 and 5 of embryo development. In this regard, due to legal restrictions a study design as suggested by Christopikou and Handyside that included the analysis of multiple samples from TE and ICM would not be possible in our country, however we are confident that such approach would not

References


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amplification rate observed in the second PB could be generally significantly different from blastomeres and TE samples. Thus, the lower other studies, the amplification efficiency of the second PB was not sig-
dataset (16.3%). Even if the diagnostic rate is indeed lower than in good prognosis patients population.

analysis of single blastomeres on good quality embryos coming from a (Handyside did not result in the predicted outcome in the corresponding zygote errors detected as copy number changes in the polar bodies that chromosomal segregation in PB1/PB2/blasto-mer and not PB1/PB2 and TE). It is also worth noting that Handyside himself found a very similar proportion (48/227; 21.1%) of chromosome segregation errors detected as copy number changes in the polar bodies that did not result in the predicted outcome in the corresponding zygote (Handyside et al., 2012). Finally, Christopikou herself (Christopikou et al., 2013) found 17% (17/100) of false-positive PB results plus 14 addition reciprocal aneuploidies in PB with normal copy number in the embryo that were considered as correct predictions without any prospective validation performed so far on PB1 log2 ratio accuracy to distinguish between whole chromosome and single chromatin aneuploidies (see comments below on this issue). However, in their upcoming paper, Christopikou et al. present their data focusing on the fact that 93% (65 of 70) of aneuploidies detected in the cleavage stage embryos were associated with copy number changes in the polar bodies giving the misleading impression that PB analysis is very accur-
ate. It seems thus that all these data further and consistently demonstrate a low specificity of PB approach in predicting the normal chromosome copy number configuration in the embryo.

Furthermore, in our study approximately half of the embryos had aneuploidies other than those arising from female meiosis. At the cleavage stage of embryo development, 25 new aneuploidies were recorded, 20 of which were confirmed at the blastocyst stage. These new aneuploidies can be equally the result of false-negative PB predictions or male and mitotic derived errors. Including these 25 de novo aneuploidies, the actual prediction of embryonic chromo-

somal complement based on PB testing decrease to 62 out of 87 aneuploidies recorded at the cleavage stage (71%) showing low sensi-
tivity of PB testing in predicting cleavage stage embryo aneuploidies. Christopikou et al. (2013) reported 7% of aneuploidies detected only in the embryo with normal segregation pattern in PB. However, in their study by Christopikou et al., the actual incidence of post-zygotic derived errors in the embryos might be significantly underestimated because of the aCGH reanalysis of the whole embryo. We would also like to point out that our results do not reflect an exceptionally mosaic set of embryos, as Christopikou and Handyside suggested, since high mosaicism rates in cleavage-stage embryos have also been observed (Vanneste et al., 2009; Mertzani
dou et al., 2013) by aCGH analysis of single blastomeres on good quality embryos coming from a good prognosis patients population.

Fragouli and Wells were also concerned about the high proportion of second PBs that failed to yield a diagnostic result, 8 of 49 in our dataset (16.3%). Even if the diagnostic rate is indeed lower than in other studies, the amplification efficiency of the second PB was not sig-
ificantly different from blastomeres and TE samples. Thus, the lower amplification rate observed in the second PB could be generally considered as a normal deviation due to the small sample size included in our dataset.

In their letter Fragouli and Wells also suggest that whole chromo-
some versus single chromatid imbalances can be readily distinguished in PB1 by aCGH log2 ratio. This is sharply in contrast with our opinion based on our own experience and on available literature, including the two papers reported by Fragouli and Wells as a proof of evidence. The study by Gabriel et al., using aCGH log2 ratio on polar bodies, found that single chromatid errors were much more common than whole chromosome errors (Gabriel et al., 2011). However, the thresholds used in this study were not validated by comparing to the outcome in the embryo (Gabriel et al., 2011). In the Handyside (2012) paper, they say: ‘Although gain or loss of whole chromosomes versus single chromatids in PB1 was apparent in some cases by the extent of the altered ratio following array CGH (especially where examples of both were present in the same plot), this was not system-
atically analysed and not considered completely reliable’. Indeed, in that study whole chromosomes versus chromatid errors in meiosis I were distinguished exclusively based on the analysis of the segregation pattern in all three products of meiosis and not solely through the PB1 aCGH log2 ratio. Furthermore, the ESHRE RCT study by itself did not include the use of the log2 ratio in PB1 to predict normal zygote due to chromatid error in meiosis I with correction in meiosis 2. It is thus our opinion that future studies are indicated to determine whether these embryos should be re-biopsied or whether the signal intensity data are reliable enough to distinguish between chromatid and whole chromosome losses and gains. These thresholds should be tested prospectively by reanalysing cases in which polar body recipro-
cal aneuploidies occurred and making blinded predictions of the chromosomal status of the embryo. At present, our recommendation on reciprocal aneuploidies in PBs is to perform a follow-up analysis in the resulting embryo.

Fragouli and Wells also raised the question of whether particular chromosomal segregation during meiosis leading to misdiagnosis based on PB chromosome screening apply also to a younger popula-
tion of patients who tend to produce a lower number of aneuploidies per oocyte. In our study based on a poor prognosis population we were able to highlight some particular meiotic segregations potentially leading to misdiagnosis based on PB chromosome screening, including anaphase lag during meiosis I and meiosis 2 and patterns consistent with monosomic oogonia due to error before entry into meiosis.

To be precise, we talked about these meiotic segregation patterns when we first presented the preliminary data of this study in 2011 (Capalbo et al., 2011a). Even if no euploid embryos have been obtained at the blastocyst stage, there is no reason to assume that the limitation of PB screening observed here is unique to abnormal oocytes and embryos of poor prognosis AMA patients. Indeed, it has recently been demonstrated in a younger population that when re-
ciprocal aneuploidy occurs from meiosis I premature separation of sister chromatids and compensation in meiosis II, the resulting embryo is usually normal for that chromosome (Forman et al., 2013) and also capable of producing a chromosomally normal child (Scott et al., 2012b). However, we agree that the reproductive poten-
tial of these embryos should be further evaluated.

Regarding the high correction rate of female-derived trisomies observed in our study, it was indeed an unexpectedly high rate. It should also be considered that the design of our study did not allow...
investigating the rate of uniparental disomies (UPDs) among corrected chromosomes and whether the UPDs were mosaic. Furthermore, our study was underpowered to estimate the actual incidence of correction events in the clinical PGS population. However, our data are well matched with a retrospective search for UPD in 77 cytogenetically normal diploid spontaneous abortions, indicating that ~2.5% of genetically unexplained miscarriages were associated with whole chromosome UPD (Fritz et al., 2001). We thus look forward to see additional data from blastocyst stage PGS cycles using higher resolution chromosomal screening tests.

Finally, despite considering the blastocyst stage approach as the gold standard in their daily clinical practice, Fragouli and Wells as well as Christopikou and Handyside point out that PB approach should be considered the best way for AMA patients at risk of producing a lower number of blastocysts. This consideration relies on the unproven concepts that the extra-manipulation during PBs biopsies does not affect embryo development and that blastocyst culture decrease implantation potential on a per cycle base (i.e. patients who do not produce blastocysts in vitro can become pregnant with day 3 embryo transfer).

On the contrary, it is our opinion that offering PB-based aneuploidy screening to patients producing few or no blastocysts may be meaningless, or even detrimental, for the following reasons:

(1) Extra-manipulations prior to blastocyst development may be potentially detrimental and lower further the embryo developmental potential. This concern may be particularly relevant for PB approach, where the manipulation is performed two times (day 0 and day 1) during a sensitive and delicate time-window (oocyte and zygote) for preimplantation embryo development. This figure is particularly relevant in the clinical setting of Christopikou and Handyside where PB approach is based on two consecutive biopsies (Christopikou et al., 2013).

(2) According to the low sensitivity of PB approach observed in AMA patients during our study (mainly due to the high rate of male or post-zygotic derived aneuploides) the PB-based aneuploidy screening may expose patients to the transfer of uniparental embryos. Testing the PBs does not exclude the occurrence of paternal and clinically relevant mitotic errors in the resulting embryos.

(3) Beside the assessment of aneuploides, it is the goal of any embryologist to avoid the transfer of developmental incompetent embryos. It has been proved that blastocyst culture do not affect embryo implantation potential on a per cycle analysis in poor prognosis patients (Guerif et al., 2009). We thus believe that transferring euploid cleavage stage embryos without monitoring their development to the blastocyst stage in vitro may increase the likelihood of implantation failure affecting subsequent reproductive history of the patients. In this scenario, and especially when patients are not producing blastocysts, the use of expensive technologies, such us CCS on PBs, for embryo selection to improve IVF success rates may be unnecessary.

To conclude, in the view of current knowledge, it is our opinion that the best approach for aneuploidy screening in preimplantation window is the blastocyst stage that gives (i) the most reliable information about chromosome complement of embryos we are going to transfer (including male and post-zygotic derived errors), (ii) no impact on developmental potential together with important information about developmental competence and (iii) the most cost-effective method for patients. Whether the accuracy achievable using polar body array comparative hybridization for PGS is good enough to improve the advanced maternal age patient population outcome remains debatable and we look forward to seeing the result of the planned randomized trial and other upcoming studies evaluating the clinical predictive value of PB-based aneuploidy screening.

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