What next for preimplantation genetic screening? Beyond aneuploidy

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Many published papers suggest a favourable impact of preimplantation genetic screening (PGS) on implantation and pregnancy rates, but more recent randomized studies have not confirmed, or could not conclude, that PGS actually improved implantation rates. Team inexperienced in embryo screening has been mentioned as the origin of the discrepancies; thus some clinicians allege a need for more powerful, well-designed, randomized studies performed by specialized teams. However, what if all the contradictory results about the benefits of PGS and implantation were not due to technical problems or team specialization but were biological in origin? The developmental programme of an eight-cell embryo relies on signals of maternal origin retrieved from the cytoplasm to initiate a new transcriptional network that will eventually serve as a filter (checkpoints and apoptosis) for aneuploidy. Thus, the use of PGS with the objective of improving the likelihood of a successful pregnancy based on nuclear abnormalities (aneuploidies) in an early cleavage stage embryo could be invalid since the information (diagnosis) obtained at the moment of biopsy could be overthrown by the transcriptional machinery of the new zygote genome.

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unethical to repeat the studies based on the existing results (Mastenbroek et al., 2008).

What if all the contradictory results about the benefits of PGS and implantation were not due to technical problems or team specialization but are biological in origin, and the result of a too strict interpretation of embryo development dynamics based on the chromosomal status of one, sometimes two, blastomeres? What is the meaning of one aneuploid blastomere in an eight-cell embryo when all eight cells may be totipotent and any one of them capable of forming an embryo? How many blastomeres have to be aneuploid before embryo development is affected, when a whole embryo can be derived from just two to three blastomeres (Barry, 2002)? What impact in the developmental programme can an aneuploid blastomere have on an eight-celled embryo in a period when the new genome is inactive and, with it, most of the mechanisms that control cell cycle checkpoints and apoptosis (Duranthon and Renard, 2003; Yanfeng et al., 2003; Los et al., 2004)? What is the influence of aneuploidy on implantation? At least 50% of embryos with aneuploidy will reach the blastocyst stage and may implant (Rubio et al., 2007) and, although most aneuploidies are incompatible with life, some can survive (trisomy 21, for example). How can an embryo be defined as normal or abnormal based on the analysis of only one or two cells, when more than half of all embryos are mosaics (Ruanguitler et al., 2000; Wells and Delhanty, 2000; Munne, 2005; Baart et al., 2006)?

As early as 2004, Los et al. (2004) pointed out that the big problem in embryo screening was not associated with technique, but with chromosomal mosaicism in the cleavage stage embryo, since mosaicism (the result of a post-fertilization mitotic error due to chromosomal mis-segregation) is present in at least 50% of zygotes analysed by PGS (Iwarsson et al., 1999; Wells and Delhanty, 2000; Munné et al., 2003; Baart et al., 2006). Needless to say, the presence of mosaicism in the embryo presents a major challenge to embryo screening; by the time the embryo biopsy is performed, the origin of aneuploidy (meiotic or mitotic) is difficult to determine unless all the blastomeres from the embryo are analysed.

In any event, the high aneuploidy rate (50%) in eight-cell embryos is reduced to 30% when aneuploidy percentages are determined in miscarriages, 20% in stillbirths and only 0.3% in newborns. From this observation it is reasonable to assume that during the process of embryonic development in the uterus, the embryo experiences a stringent self-correction probably based on cell cycle checkpoint control and apoptosis. In fact, it has been shown that loss of embryo viability due to chromosomal mosaicism is caused by the activation of a spatially- and temporally-controlled p-53 independent apoptotic mechanism and is not the result of a failure to progress through mitosis (Lightfoot et al., 2006). However, the point is that these determinant mechanisms that control programmed cell death have been documented in the blastocyst stage embryo but not in the early cleavage stage embryo (Duranthon and Renard, 2003).

Thus, if all these biological mitotic checkpoints are not operative in the biopsied embryo, is it correct to discard an eight-cell embryo because of one aneuploid blastomere? Considering that there are more possibilities that this blastomere belongs to a mosaic embryo (50%) than to an abnormal one (20%) (Iwarsson et al., 1999; Wells and Delhanty, 2000; Munné et al., 2003; Baart et al., 2006), that the actual number of aneuploid cells and the distribution of mosaicism are not known with certainty in the biopsied embryo, that the zygote stage is very permissive regarding aneuploidies and that zygotes with severe aneuploidies do reach the blastocyst stage (Rubio et al., 2007), not transferring an eight-cell embryo with one aneuploid cell deprives it of the alternatives that its new transcriptional network might offer it to develop.

Furthermore, it has been demonstrated that aneuploidies present on Day 3 post-fertilization are not found when the embryo is re-analysed 2 days later and in vitro studies analysing aneuploidies in preimplantation human development using stem cells have provided very useful information (Ambartsoumyan and Clark, 2008). For example, it has been shown that stem cell lines, derived from embryos classified as aneuploid on PGS, were karyotypically normal and none of the aneuploid lines presented the same anomaly as the original PGD analysis (Peura et al., 2008). Similar results have been reported in human stem cells derived from blastocyst-stage embryos diagnosed as aneuploid in PGS, and the authors of that study demonstrated that the euploidy in the stem cells was not achieved through chromosome duplication, so those stem cells must have originated from the mosaic embryo (Lavon et al., 2008).

These clinical and basic research findings lead us to the same question, should an eight-cell embryo, classified as ‘abnormal’, be discarded because of a single aneuploid blastomere? And the answer again is that by not transferring an eight-cell embryo we deprive it of the physiological alternatives for cellular self-correction once the new zygotic transcriptional network is operative.

Nevertheless the future of the early cleavage-stage embryo will not only depend on alterations in the cell nucleus, the number of cells with aneuploidies, or the type of aneuploidy. The chances for survival have been set even before the new genome is activated and in an eight-cell embryo they do not depend on the nucleus but on components (maternal proteins and mRNAs) that accumulate in the cytoplasm of the oocyte during oogenesis and are unleashed after fertilization (Slack, 2001; Duranthon and Renard, 2003).

Some graphic examples of the development capacity of oocyte cytoplasm capacity are: parthenogenesis (self-development of an oocyte up to the blastocyst stage without fertilization, indicating that the nucleus in those stages is not part of the developmental programme); nuclear transfer (Campbell et al., 2007), totipotent signals carried by the cytoplasm (Seydoux and Braun, 2006); and, finally, blastocyst formation in nuclear transfer is significantly higher in MII oocytes reconstructed with fresh cytoplasm than with cytoplasm from aged oocytes (Bai et al., 2009).

As reproductive specialists, we are very much aware of how important for IVF success (pregnancy) a good oocyte is and how the opposite situation (for example in women over 38) results in poor pregnancy rates, elevated number of miscarriages and high aneuploidy rates (>70%) (Yuan et al., 2002; Munné et al., 2003; Jones, 2008; Pan et al., 2008). Nevertheless such a high percentage of oocyte aneuploidies (70%) should not be used as a reason for PGS, since it is expected that they will be filtered once the mitotic checkpoints are set through a mechanism for the elimination of defective embryos once the genome is activated.

Needless to say, all the above considerations regarding nuclear and cytoplasmic dynamics apply to embryos classified as ‘chromosomally normal’ in PGS, because there are more possibilities that a normal blastomere belongs to a mosaic (50%) than an all-normal.

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(25%) embryo. If the 'chromosomally normal' zygote were a mosaic, it would be under the same controls as an embryo classified as abnormal once its new transcriptional network became operative, as explained above. Once transplanted to the uterus, an embryo that had been graded as 'normal' by the PGS test, would undergo constant sequential cleavage divisions, chromosome segregation and reprogramming, and at any moment there may be a mitotic error that will delineate its future, added to the possibility of already existing biopsy-induced damage (Mastenbroek et al., 2007).

In conclusion, the developmental programme of an eight-cell embryo (even after embryo screening) relies on signals of maternal origin retrieved from the cytoplasm to set up a new transcriptional network that will serve as a filter (checkpoints and apoptosis) for aneuploidy. Thus, if PGS is based only on nuclear information (chromosomal aneuploidies), its capacity to evaluate the implantation prognosis is limited by the fact that the diagnosis reached at the moment of biopsy may be reversed once the transcriptional machinery of the new zygote is activated during early cleavage by the effect of cytoplasmic elements. In the same vein, it seems to me that the proposal of performing powerful randomized studies with human patients to validate PGS, which studies only nuclear content, probably will not improve implantation rates because increasing the number of patients is not going to have any influence on the evolutionarily-determined cytoplasmic signals controlling embryo development. Randomized studies focused on the possible effects of taking one or two blastomeres and/or the impact of biopsy on embryo survival on implantation will not affect the cell cycle checkpoints that regulate growth arrest, DNA repair and programmed cell death and thereby prevent the formation of aneuploidic cells. The PGS biopsy may damage the structure of the zygote, but it is not going to alter the intracellular cytoplasmic signals originating in the maternal cytoplasm that control early embryo development.

Respecting the main question of this debate: what next for PGS? In my opinion, PGS, based on the latest clinical results, developmental data and scientific society recommendations (Practice Committee of the ASRM, 2007), does not seem to be a tool that can really increase implantation rates in our patients.

References


