two cells, instead of one, from cleaved embryos. The fact that the outcome was rather disappointing is not surprising since it was already shown in two previous reports that removing two cells from cleaved embryos on day 3 can be akin to harming the embryo beyond repair (Bahçe, 2003; Magli et al., 2004). In spite of what the authors assert, it is not the rate of unaltered blastocyst development that occurs as a result of the removal of one or two cells that is important. Rather, it is the fact that the portion of cells in the inner cell mass is diminished when biomass is removed from early embryos proportionately. We strongly suggest that the group in Brussels revisits this important discussion, since their presentation is, at best, confusing. In applying such an invasive procedure as biopsy in a laboratory that only has an implantation rate of 11% when blastocysts are replaced, it is not surprising that the biopsy—regardless of number of cells removed—harms the embryo’s further development. Fortunately, the strength of PGD’s selection process compensates for this procedure’s related detriment in the Staessen study, strengthening, and not weakening, as the author’s contend, the ability of chromosome analysis to compensate for the already artificially induced injury. In years to come, this report will probably be hailed by opponents of PGD for infertility. Let it be said here that this study, in spite of its good intentions, particularly with its use of randomization, does not prove that aneuploidy testing in cleaved embryos, using a restrictive number of probes for chromosomes, does not work. On the contrary, it shows that the test is helpful, even when conditions are suboptimal.

References

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Reply to Comments on Staessen et al. (2004)

Sir,

We appreciate the interest of Mastenbroek and colleagues, the implantation rate is an inappropriate measure since the number of embryos transferred depends on the strategy and not on the design. The transfer policy is mentioned in Materials and methods and the results presented are valid for the transfer policy followed. What complicates the situation of course is that different numbers of embryos were transferred between the two groups, although this was not intentional. As we discussed in the paper, our trial might have given different results if an equal number of embryos were transferred in both groups. However, this would have been a difficult and even unethical design given the unpredictable numbers in this subgroup of patients. Eventually a design in which only one embryo would have been transferred in both study arms could have been chosen, but is it ethical to transfer only one embryo in patients above 37 years of maternal age if more are available, especially in the control group? Nevertheless, the study, as it was conducted, has led us to conclude that this RCT provides no arguments in favour of PGD in patients with advanced maternal age (AMA) when there are no restrictions in the number of embryos to be transferred. The allocation of the patients to the study was not double-blinded because it was felt unethical that patients would not be aware of what treatment their embryos would be exposed to. At the level of embryo selection, Mastenbroek et al. express their doubts about the blinding. However, we can reassure them that the blinding was effective: in the IVF laboratory, the embryos are evaluated and judged to be transferable on the basis of morphological characteristics by an independent embryologist and, among those embryos, the genetically normal are transferred.

All relevant data are available in the manuscript allowing recalculations. The results as recalculated by Mastenbroek et al. show an ongoing pregnancy rate of 11% in the PGS group versus 15% in the control group [relative risk (RR) 0.72, 95% confidence interval (CI) 0.43–1.21]. The conclusion of the RCT study thus remains the same, i.e. that in terms of clinical pregnancy rate there is no difference between the control group and the PGS group. In view of this recalculations, the conclusion of our RCT that the effectiveness of PGS to improve IVF/ICSI in women with AMA, at least when there are no restrictions on the number of embryos for transfer, remains unproven, is still valid.
The issue raised by Cohen and Munné brings us to a more delicate point that has been a target of discussion for a long time. The biopsy of one blastomere may be less detrimental for the further development of the embryo, but may jeopardize the accuracy of the diagnosis. The references mentioned by Cohen and Munné are not convincing: Bahçe (2003) is comparing 383 embryos from which in 99% (379) one blastomere was removed and in 1% (four) two cells were removed. The implantation rate was 41.17% after one blastomere biopsy versus 18.6% in the two-blastomere biopsy. The reference of Magli et al. (2004), which actually does not deal with the comparison of one- and two-cell biopsies, indeed mentioned in the Introduction a retrospective analysis of data: 29% implantation after one-cell biopsy versus 16% after two-cell biopsy. No other data, such as the number of embryos analysed, are presented. Cohen and Munné infer that the low implantation rate after one-cell biopsy versus 16% after two-cell biopsy. No other data, such as the number of embryos analysed, are presented. Cohen and Munné infer that the low implantation rate of blastocysts was due to suboptimal culture conditions, an argument that overlooks the mean age of the patients included and their unfavourable infertility history. Also, there were no exclusions because of higher day 3 FSH, failure on previous attempts or insufficient follicles during stimulation. In a typical European setting with IVF reimbursement, implantation rates tend to be lower because of the lack of selection criteria that tend to include ‘optimal’ patients.

References


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The predictive value of sperm chromatin structure assay

Sire,


Perhaps, before discussing the predictive value of the sperm chromatin structure assay (SCSA) test in assisted reproductive technologies, it would be useful to review the causes and types of DNA damage found in human sperm and the different tests that can be used to assess this damage.

DNA damage in sperm can affect both mitochondrial and nuclear DNA and can be induced by five main mechanisms: (i) apoptosis during the process of spermatogenesis; (ii) DNA strand breaks produced during the remodelling of sperm chromatin during spermiogenesis; (iii) post-testicular DNA fragmentation induced by oxygen radicals, including the hydroxyl radical and nitric oxide, during sperm transport through the seminiferous tubules and epididymis; (iv) DNA fragmentation induced by endogenous endonucleases; and (v) DNA damage induced by radio- and chemotherapy. Of these five mechanisms, perhaps the one that may play a major role in causing sperm DNA fragmentation is post-testicular damage induced by oxygen radicals during sperm transport. This is supported by previous reports demonstrating that DNA fragmentation is higher in epididymal (Steele et al., 1999) and ejaculated (Ollerø, et al., 2001; Greco et al., 2005) compared to testicular sperm.

DNA fragmentation induced by the hydroxyl radical results in the formation of 8-OH-guanosine and 8-OH-2′-deoxyguanaine in a first stage followed by double-stranded DNA fragmentation thereafter (Cui et al., 2000). While DNA damage of the first type could be repaired to some extent by the oocyte, double-stranded DNA damage is irreversible and incompatible with normal fertilization and the development of a viable pregnancy. Since DNA fragmentation values in ejaculated human sperm >10%, as assessed by TUNEL (Benchab et al., 1993), or >30%, as assessed by the SCSA test (Evenson et al., 1999), are associated with low pregnancy rates, one would think that the remaining 90 and 70% of the sperm respectively could fertilize the oocyte and result in a viable pregnancy. However, in addition to double-stranded DNA breaks, a significant proportion of these sperm could have DNA base modifications of the 8-OH-guanosine and 8-OH-2′-deoxyguanaine type. Therefore, the probability that a spermatozoon with normal DNA would fertilize the oocyte would be much lower than that expected from a DNA fragmentation value of 10 or 30% respectively. That is, in addition to the measurable 10 and 30% of sperm with DNA fragmentation, the remaining 90 and 70% of sperm would have some type of DNA damage that is not compatible with the development of a viable pregnancy. This concept has been designated as the ‘iceberg effect’ (Evenson et al., 1999). If, on the other hand, DNA damage is related to single-stranded DNA breaks, like those produced during the process of chromatin remodelling, this type of damage would not be normally expressed after sperm fertilization, since it would require dissociation of both DNA strands during sperm decondensation and male pronuclei formation. Tests such as the SCSA, DNA breakage detection (DBD)–FISH (Fernandez et al., 2000), SCD (Fernandez et al., 2003),...