Detection of Chromosomal Abnormalities in Human Preimplantation Embryos Using FISH*

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Purpose: Multicolour FISH has been used for the preimplantation diagnosis of sex for X-linked disorders and to examine the chromosome constitution of early human embryos.

Materials and Methods: Single blastomeres and whole embryos were spread using HCl and Tween 20. Multicolour FISH was performed using directly-labelled human DNA probes for chromosomes X, Y, and 1 in a two hour FISH procedure.

Results: Four groups of chromosome arrangements have been found in human preimplantation embryos (i) normal, all nuclei uniformly diploid, (ii) diploid mosaics, majority of the nuclei diploid, with a small number of nuclei aneuploid (iii) chromosomally abnormal, all nuclei uniformly chromosomally abnormal, e.g. XO, XXY, XXX and (iv) chaotic, all nuclei showing different chromosome complements.

Conclusions: For the preimplantation diagnosis of sex, an XX nucleus has always been representative of a female embryo. However, for the diagnosis of dominant disorders or chromosome abnormalities, two cells should be analysed to reduce the chance of misdiagnosis which may arise from chromosomal mosaicism. Implantation and further embryo development may be possible from mosaic or chromosomally abnormal embryos, but those showing chaotic chromosome arrangements would be unlikely to implant.

KEY WORDS: multicolor FISH; preimplantation embryos; mosaicism; chromosomal abnormalities.

Since 1991 we have used fluorescent in situ hybridisation (FISH) to sex human embryos for preimplantation diagnosis of X-linked diseases. The advantage of FISH over molecular methods such as the polymerase chain reaction (PCR) is that the exact number of chromosomes present can be easily visualised, as well as problems with contamination being eliminated.

Initially we performed dual FISH with probes for chromosomes X and Y using conventional spreading (methanol:acetic acid) (15). This method of spreading proved fairly reliable for single cells (4), but during the early stages of any diagnosis it is important to examine those embryos not transferred to the patient to confirm the diagnosis and learn more about embryo development. Using methanol:acetic acid spreading on whole embryos proved less successful, and in most cases only one or two cells from the spare embryos were analysed (5). During these initial studies we detected some chromosomal abnormalities, such as XO, XXX, and XXY nuclei, and an XO embryo was identified which has important consequences for those patients carrying X-linked disorders (2). With the use of a more refined spreading method developed in mouse embryos using HCl and Tween 20 (1), which could be successfully used on human blastomeres (7) and whole embryos (8), it became possible to examine more of the nuclei in a whole embryo using FISH.

Since we had detected chromosomal abnormalities during our preimplantation diagnosis programme, it was important to assess the level of such abnormalities to ensure that they would not lead to a misdiagnosis of sex or other diagnoses currently being developed. We therefore used dual-colour FISH with either the sex chromosomes or autosomes 1 and 17 on a series of embryos donated for research purposes (9). For the sex chromosomes and autosomes, when the whole embryo was examined, a high level of chro-
mosome abnormalities was detected (Table I). In the majority of cases the embryos were diploid mosaics, whereby most cells were diploid, with between one and three cells being aneuploid. In a few cases, especially when examined for autosomes, every nucleus within an embryo was abnormal, indicating chaotic chromosome division.

Even with the high level of chromosomal abnormalities detected, for the diagnosis of sex an XX nucleus has always been representative of a female embryo, but interestingly XO nuclei are present in both male and female embryos. However, with the development of diagnosis for chromosomal abnormalities such as translocation carriers and dominant disorders, haploid or aneuploid nuclei within a diploid embryo could lead to a misdiagnosis (3,6). For example, we are currently performing preimplantation diagnosis on four patients at risk of transmitting unbalanced chromosome complements (Conn et al., in preparation). In one case where the patient is a suspected gonadal mosaic for chromosome 21, on day 4 postinsemination we detected two disomic cells in an embryo otherwise trisomic for chromosome 21. To reduce such a problem to a minimum, we intend to transfer only embryos where two nuclei have been shown to be normal diploid. We are also currently developing a PCR procedure for the detection of a mutation leading to the development of adenomatous polyposis coli, an inherited colon cancer predisposition syndrome. This is a dominant disorder and so the presence of haploid nuclei containing only the unaffected allele could lead to a misdiagnosis. Again, the results of two nuclei would reduce this problem to a minimum.

Since human embryos show high levels of mosaicism (9,13,14), we now use triple-colour FISH for preimplantation diagnosis of sex to provide more information on the status of the biopsied cell and examination of the spare embryos (11). For the diploid mosaics, the use of an autosomal probe allows the distinction between diploid/monosomy and diploid/haploidy, etc. In a series of five cycles, we found that the majority of diploid mosaics was diploid/haploid, but diploid/tetraploid mosaics have also been observed (Delhanty et al., in preparation). Both of these cases can be explained by a failure of normal mitosis. As with our previous studies on embryos donated for research purposes, and from our preimplantation diagnosis programme, we also detected a number of embryos showing chaotic chromosome arrangements. The basis of this uncontrolled division is unknown, but in our preimplantation diagnosis programme it seems to be patient related since almost all the chaotic embryos were from two patients. If relatively few cells of an embryo show an abnormality, it is possible that such cells may be selected to form the trophectoderm and not the embryo proper (12), but it seems very unlikely that embryos showing totally chaotic chromosome constitutions could develop normally.

The availability of human embryos to examine these phenomena further is limited due to ethical constraints. However, two groups of patients exist: those infertile patients undergoing routine IVF who may donate their embryos for research and those mainly fertile patients undergoing preimplantation diagnosis. Differences may exist between the two groups. For example, work to date has indicated that for patients undergoing preimplantation diagnosis, embryo grade bears no relation to chromosome abnormality, which does not seem to be the case for infertile patients. Further studies using multicolour FISH on embryos from these two groups of patients may lead to important information in the study of human preimplantation development (10). In general, the chance of conception in any one ovulatory cycle is low, and for women under 35 years there is an approximately 25% conception rate per cycle whether they conceive naturally or via IVF. This cannot be explained by age-related aneuploidy, and hopefully the use of techniques such as FISH will help unlock the "black box" concerning events between conception and implantation.

**REFERENCES**
