Success Rate

The mean success rate per cycle is about 10%; it decreases with the number of insemination cycles, from 10.3% during the first 6 months to 2.3% after 24 cycles of treatment. This indicates that more fertile women conceive more readily. This success rate is dependent on the women's ages, the AID indication, the semen quality, and especially, the postthaw motility. The cumulative success rate (dropout excluded) for all women is 48% of pregnancies at 6 cycles, 66% at 12 cycles, and 80% at 24 cycles. The mean success rate per cycle is 15% with intrauterine insemination and 23% with IVFD (Table III).

Pregnancy Outcome

Since 1987 the CECOS Federation has implemented a prospective study on pregnancies achieved by AID (Table IV). The data concern 11,800 pregnancies (2.3% lost to follow-up). The miscarriage rate is 17.7%. The rate of ectopic pregnancies is 0.8%, and 81.1% of the pregnancies were continued to delivery; among these, 95% were single, 4% twins, and 0.4% triplets. The sex ratio was normal and the overall incidence of malformations (including Down syndrome), less than 2%, is lower than in the general population.

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Preimplantation Diagnosis of Genetic and Chromosomal Disorders

INTRODUCTION

Since preimplantation genetic diagnosis (PGD) was first undertaken a few years ago (1–4), nine centers in different areas of the world have started applying this new technique clinically and have already assisted dozens of couples at risk of producing offspring with genetic disorders to become pregnant and deliver children free of genetic disorders (5,6). Recent experience was reviewed by the Fourth Annual Meeting of the International Working Group on Preimplantation Genetics, organized in association with the international symposium, "Genetics of Gametes and Embryos," New York, June 2–5, 1994. The scope of the meeting included the current status of PGD, existing problems in sampling and genetic analysis, and areas for research in preimplantation genetics.

Overall, 116 at risk couples participated in the clinical trials currently ongoing in these centers, and of a total of 164 PGD cycles performed to date, 124 resulted in embryo transfer, 42 in a clinical pregnancy, and 31 in the birth of an unaffected child (Table I). Most centers performed a cleavage-stage PGD and achieved pregnancy rates comparable to that in standard IVF practice (20–30%). PGD for gender determination was performed in about half, and for single gene disorders in about a third, of the cycles; PGD clinical trials have been also initiated for common chromosomal trisomies (25 cycles), raising the possibility for wider application of PGD in assisted reproduction practices for couples of advanced maternal age.

It is now important to develop and improve the methods for sampling and genetic analysis not only to make PGD more efficient and safe, but also to avoid possible misdiagnoses, reported to date by three most experienced centers (7–9).

Table I. Global Experience with PGD[a]

Genetic condition	Patients	Cycles	Transfers	Pregnancies	Babies born
Gender determinators for X-linked disorders	54	83	70	23	21
Single-gene defects	38	56	49	14	9[b]
Chromosomal disorders	24	25	15	5[c]	1
Total	116	164	134	42	31

[a] Includes data reported from eight centers: Hammersmith, Cornell, Chicago, Brussels, Fairfax, Norfolk, Barcelona, and Adelaide.
[b] One affected.
[c] Four ongoing pregnancies.

PREIMPLANTATION DIAGNOSIS FOR X-LINKED DISEASES

Risk for X-linked disease was one of the major indications for preimplantation diagnosis at most centers (Table II). Initially, this was performed using cleavage-stage biopsy and DNA amplification of Y (1)-, and then both X- and Y-specific sequences by polymerase chain reaction (PCR) (3). Later, fluorescent in situ hybridization (FISH) with X and Y chromosome-specific probes was used (10–12). In addition, the use of an 18-chromosome specific probe in combination with X and Y probes has been recommended to minimize misdiagnosis due to mosaicism (13). A total of 54 patients was studied (28 by DNA amplification and 26 by FISH) in 83 cycles, resulting in 70 transfers and 23 pregnancies, with 21 unaffected live-born children. Because of the possibility of misdiagnosis when DNA amplification is used at the cleavage stage, as reported in one of the first clinical cases by the Hammersmith group (7), preimplantation sex determination is currently performed by FISH at most centers. Although some groups still continue to use highly improved methods of DNA amplification such as a single set of primers amplifying the homologous ZFX and ZFY sequences in the X and Y chromosomes (14) or X- and Y-linked amelogenin genes combined with the repetitive DYZ1 Y-linked satellite marker (15,16), a strong opinion is evolving that preimplantation gender determination should be done by FISH.

Mosaicism and sperm DNA contamination may be possible problems of the cleavage-stage gender determination. The Hammersmith and Cornell groups have observed high rates of mosaicism in cleavage-stage human embryos involving both sex chromosomes (12,17) and autosomes (17,18). Obviously, sex determination by PCR of a single blastomere from an embryo with XO or XY/XO mosaicism may lead to misdiagnosis of an XO blastomere as female (unaffected) and may result in the birth of a child with Turner's syndrome at high risk for X-linked disorder. The limitations of PCR for gender determination at the cleavage stage have been also demonstrated by the Cornell (19) and Chicago groups (unpublished data), using a combination of PCR and FISH techniques for PGD of X-linked diseases. Munné and collaborators (19) reported the use of FISH analysis in four clinical cases following PCR, either when PCR failed or when embryos were diagnosed as males (to confirm the diagnosis) or arrested in the development. In two of these cases, in addition to the embryos selected for transfer by PCR, one embryo in each case was diagnosed by FISH. The transfer resulted in the birth of two normal twin females in one of these cases. In the confirmatory analysis of the arrested embryos or those diagnosed as affected by PCR, although FISH data were in agreement with the sex of the embryos di-

Table II. PGD for X-Linked Disorders by Gender Determination[a]

Method of genetic analysis	Patient	Cycles	Transfers	Pregnancies	Babies born
PCR	28	46	39	13	11
FISH	26	37	31	10	10
Total	54	83	70	23	21

[a] Includes data reported from six centers: Hammersmith, Cornell, Chicago, Brussels, Fairfax, and Barcelona.

agnosed by PCR, FISH analysis detected two mosaic embryos and two abnormal embryos, XO and XXY, diagnosed by PCR as normal female and male. Verlinsky and collaborators (unpublished data) used a combination of FISH and PCR for the cleavage-stage PGD of hemophilia A in one clinical case. Two blastomeres were biopsied from each eight-cell embryo available for genetic analysis, one being used for DNA amplification and the other for FISH. Only those two embryos which appeared to be unaffected females based on both methods were transferred. Three embryos diagnosed as female by PCR appeared to be either XO, XX/XY, or XY/XYY by FISH, suggesting possible amplification of sperm DNA or allele-specific amplification failure in the latter two and amplification of an XO blastomere in the first. Therefore, in spite of the limitations of FISH described under Problems in Accuracy (below), FISH alone or in combination with PCR seems to be a reliable approach for preimplantation gender determination and probably will be the method of choice for PGD of X-linked disorders at the cleavage stage.

PRECONCEPTION AND PREIMPLANTATION DIAGNOSIS OF SINGLE-GENE DEFECTS

Most centers have also attempted PGD by PCR for single-gene defects (Table III). A total of 56 PGD cycles has been carried out in 38 couples, embryos transferred in about 90% of the cycles, resulting in 14 pregnancies, 7 births of children free of the genetic conditions in question, and 2 misdiagnoses, leading to selective termination of pregnancy in 1 case. The cystic fibrosis (CF) ΔF-508 mutation was the major indication for PGD for single-gene defects: 33 cycles were carried out in 27 couples, which resulted in 31 embryo transfers, 8 pregnancies, and 5 unaffected children born. Both misdiagnoses were in this group (8,9). In both of these cases cleavage-stage PGD was performed for couples in whom only one partner carried the ΔF-508 mutation, the other having a different CF mutation. That is, the potential affected fetus would be a compound heterozygote. Selective termination was performed in one pregnancy, but the second went to term despite a prenatal diagnosis that identified the pregnancy as affected. Among the other indications were for following: (i) Tay–Sachs disease—three patients, five cycles, three transfers, and one pregnancy resulting in the birth of an unaffected child; (ii) Lesch–Nyhan syndrome (LN)—two patients, four cycles, three transfers, and one pregnancy with delivery of an unaffected child; and (iii) hemophilia A—two patients, six cycles, six transfers, and one pregnancy resulting in a spontaneous abortion. Also, for (iv) Duchenne muscular dystrophy (DMD), (v) α-1-antitrypsin deficiency, (vi) retinitis pigmentosa, and (vii) fragile X, there was a single patient per condition, involving a total of eight cycles and six transfers, resulting in two ongoing pregnancies (DMD; fragile X). Genetic analysis in most cases involved the development of a specific strategy based on extensive preliminary studies: Examples are PGD of LN syndrome (Hammersmith), DMD (Brussels), Fra-X (Fairfax), CF ΔF-508 (Adelaide), and retinitis pigmentosa (Chicago).

As mutations causing the deficiency of hypoxanthine phosphoribosyl transferase (HPRT) in LN syn-

Table III. PGD for Single-Gene Defects[a]

Condition	Patients	Cycles	Transfers	Pregnancies	Babies born
Cystic fibrosis	27	33	31	9	6[b]
Tay–Sach's disease	3	5	3	1	1
Lesch–Nyhan syndrome	2	4	3	1	1
Duchenne's muscular dystrophy	1	1	1	1	1
Hemophilia	2	6	6	1[c]	
α-1-Antitrypsin	1	5	3	0	
Retinitis pigmentosa	1	1	1	0	
Fragile X	1	1	1	1	
Total	38	56	49	14	9

[a] Includes data reported from seven centers: Hammersmith, Cornell, Chicago, Brussels, Fairfax, Norfolk, and Adelaide.
[b] One affected.
[c] Spontaneous abortion.

drome are heterogeneous, Handyside and Hughes and their colleagues (unpublished data) first sequenced the mutant gene and developed an individual nested PCR and mutation detection method for each of their two patients. A pregnancy was established in one patient on the third attempt, resulting in the birth of an unaffected girl after confirmation of the diagnosis by CVS.

A specific PCR assay for identifying male embryos carrying a deletion of exons 13 to 18 causing DMD was developed by the Brussels group (Liebaers and collaborators, unpublished). A preimplantation diagnosis was performed in a couple at risk based on the presence or absence of exon 17. Intracytoplasmic sperm injection (ICSI) was performed in metaphase II oocytes from the carrier, and the resulting embryos were sampled at the cleavage stage. Of six embryos tested, four appeared to be unaffected, and three were transferred, resulting in a singleton pregnancy. Amniocentesis at 16 weeks showed a normal noncarrier female and unaffected infant was born.

A first PGD for *fragile-X syndrome* was reported by the Fairfax group (Levinson and collaborators, unpublished data). PGD was performed at the cleavage stage, using nested PCR detection of CCG repeats combined with multiplex PCR for gender determination. The resulting pregnancy is ongoing, but the diagnosis was not confirmed.

Matthews and Cui (the Adelaide group, unpublished data) performed cleavage-stage PGD in two cycles by direct PCR detection of a 3-bp deletion at position 508 of the CF transmembrane regulator gene. Of 14 biopsied embryos in the first cycle, 2 unaffected embryos were identified and replaced. A singleton pregnancy was achieved, but it aborted spontaneously at 7 weeks.

One child has been born following PGD for Tay–Sachs disease by the Norfolk group (unpublished data). The approach used was similar to that developed by the Chicago group (8), involving nested PCR and heteroduplex formation for a 4-bp insertion in exon 11 of the *hexosaminidase gene*. Three of seven biopsied embryos were diagnosed as unaffected and transferred, resulting in a singleton pregnancy. The diagnosis was confirmed at amniocentesis and a normal female infant was born.

PROBLEMS IN ACCURACY

The collected experience of PGD for single-gene defects shows that the major problem to be resolved remains avoidance of misdiagnosis. As mentioned, two misdiagnoses have been described following cleavage-stage PGD for CF. These might be explained either by sperm DNA contamination or by failure of allele-specific amplification. The latter possibility may be studied for each gene locus in question before PGD is undertaken. A possible contamination with sperm DNA can be avoided using microsurgical fertilization by intracytoplasmic sperm injection: this approach has been used routinely by the Brussels group (20). Substitution of ICSI for routine insemination as in current IVF practice may be useful in cleavage-stage PGD for single-gene defects.

Given the above, preconception diagnosis of single-gene defects by polar-body sampling could be reasoned to be the method of choice if sperm DNA contamination is confirmed to be a problem. According to the experience reported by the Chicago group (8,21), polar-body-inferred PGD appeared to be accurate in predicting genotype and selecting unaffected oocytes for fertilization and transfer. The low pregnancy rate reported following polar-body diagnosis could have reflected the low mean number of embryos available for transfer, rather than a detrimental effect of polar-body sampling. This was demonstrated in a follow-up study of embryos resulting from polar body-sampled oocytes in a clinical trial of PGD of common chromosomal trisomies related to maternal age (see below).

Some recent developments in DNA analysis of single cells may also be highly relevant for improving the efficiency and accuracy of PGD for single-gene defects. The major limitation of PGD using single blastomeres or polar bodies is the limited genetic material available in a single cell: This restricts the number of analyses needed for a confirmation, simultaneous amplification of several loci, and backup analysis in multiple aliquots. The increasing potential of primer extension preamplification (PEP) for single-cell DNA analysis has been demonstrated recently. This involved whole-genome amplification using random 15-base oligonucleotides as primers (22,23). Arnheim and collaborators (unpublished data) have shown the possibility of accurate genotyping of single sperm using aliquots as small as 1 to 5 μl of the PEP product. However, before this method can be applied for PGD of a specific single-gene defect, locus-dependent variation in amplification efficiency must be investigated.

PCR coupled with automated fluorescent detec-

tion of the PCR product by a highly sophisticated gene scanner also shows great potential for increasing the reliability of genetic analysis in single cells (24). Findlay and collaborators reported using this rapid and highly discriminative automated test for single-cell diagnosis of CF mutations, with simultaneous sexing and highly informative DNA fingerprinting to exclude DNA contamination (unpublished data). The possibility of quantitative analysis by this method is being explored, to investigate the possibility of simultaneous identification of common aneuploidies in the same electrophoretic lane.

Another new method of genetic analysis, considered by Hughes and collaborators to have great potential for PGD in the future (unpublished data), is comparative genomic hybridization (CGH), originally developed for molecular cytogenetic analysis of tumor cells (25). It involves simultaneous hybridization of both biotinylated total tumor DNA and dioxigenin-labeled genomic reference DNA to normal metaphase spreads, followed by quantitative measurement of the ratio of green-to-red fluorescence, reflecting the relative amounts of tumor and reference DNA at a given chromosomal locus. The usefulness of the method in PGD is obvious, as it may allow rapid overview and detection of regions of the genome containing specific mutant genes.

An approach to combined PCR and molecular cytogenetic methods has been explored by Thornhill *et al.* (26). The procedure, which they call recycling, involves FISH analysis for chromosomal aneuploidy, following PCR to detect single-gene defects, in the same cell fixed to a small piece of glass. Of 57 mouse blastomeres studied for amplification of the β-globin gene followed by FISH analysis of X and Y chromosome-specific signals, 70% amplified, and 75% of these showed detectable sex chromosome signals similar to those in the control samples not exposed to PCR. Similar work on human polar bodies and blastomeres is under way by the Chicago group (unpublished data).

Because an increasing number of severe genetic disorders has been shown to be due to extensive expansion of triplet repeats, Holding and Monk (unpublished data) have explored the possibility of reliable identification of such mutant alleles in single cells. Using DNA or cells from normal and fragile-X individuals, they have developed a method that correctly identified 95% of normal and 84% of fragile-X samples.

Finally, three centers have reported attempts to detect preimplantation gene expression, which might be useful in the future for functional assessment of the genotype of oocytes and preimplantation embryos. Gore-Langton and collaborators (unpublished data) have tested the feasibility of detecting transcripts of the developmentally relevant *connexin43* gene in normal human cleavage-stage embryos and demonstrated the presence of these transcripts as early as the four-cell stage. Verlinsky and colleagues (27) investigated homeobox genes in human oocytes and embryos at different stages of preimplantation development and also detected transcripts of homeobox genes in both cleavage-stage embryos and oocytes. *HoxA4* was found in normal and unfertilized oocytes, as well as in the four-cell embryo, while mRNA for *HoxA7* was present in normal oocytes and triploid cleaving embryos. Daniels and Monk (unpublished data) have shown the transcripts of the paternally inherited myotonic dystrophy gene in human one- to four-cell-stage embryos. These data show the feasibility of developing methods for detecting transcriptionally relevant abnormalities during preimplantation development.

PRECONCEPTION AND PREIMPLANTATION DIAGNOSIS OF CHROMOSOMAL DISORDERS BY FISH

The feasibility of PGD for chromosomal disorders has already been demonstrated by the Chicago, Hammersmith, and Cornell groups (12,13,18,28, 29).

The Chicago group concentrated on developing approaches for polar body-inferred chromosomal analysis and reported methods for visualizing second polar-body chromosomes in a state suitable for cytogenetic analysis (28,29). Reliable results were also obtained using FISH analysis of the first polar body. In a series of 130 oocytes, the number of X chromosome- and chromosome 18-specific signals were analyzed in both the second meiotic metaphase and the corresponding first polar body (using directly labeled fluorescent α-satellite DNA probes); five nondisjunction events were detected, involving either an entire chromosome or one of the chromatids. FISH analysis has been also applied for cytogenetic evaluation of the second polar body and chromosome-specific signals have consis-

tently been demonstrated in the second polar-body interphase, suitable for detecting and avoiding the transfer of embryos with common chromosomal trisomies.

The Hammersmith group applied FISH for cytogenetic analysis of embryos diagnosed as cytogenetically affected in clinical cases when PGD was done for sex selection and, also, in normally fertilized donor cleavage-stage embryos. They found that chromosomal abnormalities are a common feature of preimplantation embryos (12). Of 152 embryos studied by FISH using X- and Y-specific probes during PGD, 17 appeared to have sex-chromosome aneuploidy or mosaicism (30). In another series of 69 spare embryos, chromosome-specific probes for either X and Y or autosomes 1 and 17 were used (31). For the sex chromosomes, 29 were normal diploid and 5 (15%) were mosaic. In no case was an XX nucleus found in an XY embryo, therefore such mosaicism would not lead to misdiagnosis of sex. For the autosomes, 1 embryo was triploid, 1 was monosomic for chromosome 1, and 10 others were diploid/aneuploid or polyploid mosaics.

These data and also the results obtained by the Cornell group (13,17,18) suggest, that the high frequency of chromosomal abnormalities found in cleavage-stage embryos is not related to the FISH technique, but reflects the actual chromosomal constitution of the cells of the cleaving embryos obtained by IVF. Munné and collaborators performed FISH analysis in cleavage-stage embryos in relation to their morphological abnormalities and maternal age, using four chromosome-specific probes simultaneously (13/21, 18, and X and Y probes). Of about 400 cleavage-stage embryos studied, 49% were morphologically abnormal (34% slow or fragmented and 15% arrested embryos). FISH failure was estimated to be 7.2% for the 13/21 and 2.7% for the 18, X, and Y chromosome-specific probes; there was a significant increase in polyploidy and mosaicism in slow and arrested embryos. Polyploidy was observed in only 3.6% of normally developing embryos, in contrast with 12% of the slow and 46% of the arrested embryos. Mosaicism was most frequent (19.5%) in the slow embryos, compared with 3% of the normal and 10.7% of the arrested embryos. A direct relationship was observed between the frequency of aneuploidy and the age of the patients. Neither this nor the correlation of the frequency of chromosomal abnormalities with the developmental anomalies can be explained by the limitations of the FISH technique.

Although many chromosomally abnormal embryos, especially autosomal monosomics, will not develop further and implant, the biological significance of the high frequency of mosaicism observed at the cleavage stage remains unclear. It is possible that chromosomally abnormal lines in four- to eight-cell embryos have a detrimental effect and are consequently eliminated or that the abnormal cells selectively aggregate in the developing trophectoderm. However, it is clear that in some cases blastomere analysis at the cleavage stage will not be representative of the whole embryo. A clinical trial has been initiated by the Cornell group (unpublished data), to apply FISH for detecting and avoiding transfer of embryos with trisomy 13/21, 18, and X and Y in IVF patients of advanced maternal age. To date eight IVF patients have been offered this possibility, three of nine cycles have resulted in an clinical pregnancy and one in a healthy delivery (Table IV). A similar trial involving analysis of the first and second polar bodies is being carried out by the Chicago group (unpublished data). Five of 16 IVF cycles resulted in embryo transfer, and 2 in an ongoing pregnancy. Because of the limitations of PGD for chromosomal disorders at the cleavage stage, preconception diagnosis may be particularly valuable for detection and avoidance of the transfer of chromosomally abnormal embryos in IVF patients of advanced maternal age.

Table IV. PGD for Chromosomal Disorders[a]

Approach for PGD	Patients	Cycles	Transfers	Pregnancies	Babies born
Cleavage stage	8	9	10	3	1[b]
Polar body	16	16	5	2	0[b]
Total	24	25	15	5	1

[a] Cleavage-stage cases from the Cornell group; polar-body cases from the Chicago group.
[b] Two ongoing pregnancies.

CONCLUSIONS AND RECOMMENDATIONS FOR INITIATING PROGRAMS

A number of recommendations from the meeting might be useful for those centers considering a possible organization or application of PGD to clinical practice. First, PGD should be based on an active IVF clinic with reasonable pregnancy and take-home baby rates. All patients should be adequately counseled to ensure that the couples entering the program realize the present status of PGD, its safety, accuracy, and efficacy, and the need for confirmation by CVS or amniocentesis.

Depending on indication, different strategies for performing PGD may be recommended. It has become clear, for example, that the best approach for PGD of X-linked disorders by gender determination is FISH analysis of both X and Y chromosome-specific sequences.

For PGD of single-gene defects, to avoid a possible contamination with sperm DNA, genetic analysis may be done either on the first polar body or by cleavage-stage PGD following ICSI. The use of PEP is recommended to be able to amplify several loci and include internal controls or to repeat DNA analysis for the condition in question. This should involve prior testing of locus-dependent variation of the amplification efficiency, to exclude a possible dropout of the allele in PEP. Exclusion of couples at risk for producing double-heterozygous offspring is recommended at the present time. If the partners carry different mutations, as in the CF cases mentioned above that resulted in misdiagnosis, each mutation should be followed at all steps of the PGD, to avoid allele-specific amplification failure.

For PGD of chromosomal disorders, FISH analysis of single blastomeres may not be representative of the whole embryo because of the high frequency of chromosomal mosaicism at the cleavage stage. Before the biological significance of this phenomenon is understood, cytogenetic evaluation of the first and/or second polar body could be useful for detecting and avoiding the transfer of embryos with common trisomies in IVF patients of advanced maternal age.

Although dramatic recent developments in molecular genetic analysis of single cells will make PGD more accurate and reliable in the near-future, these highly sophisticated techniques should be applied with extreme caution before they have been experimentally tested using appropriately designed protocols.

Finally, there is a great need for international data collection on PGD cases (5). Follow-up of the outcomes and long-term surveillance of the health status of the children born following PGD are strongly recommended. All centers which perform PGD are invited to send their data to the Working Group on Preimplantation Genetics, which will be responsible for organization of the collection and dissemination of the material to participating PGD centers.

REFERENCES

1. Handyside AH, Kontogianni EH, Hardy K, Winston RML: Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344: 768–770
2. Verlinsky Y, Ginsberg N, Lifchez A, *et al.*: Analysis of the first polar body: Preconceptual genetic diagnosis. Hum Reprod 1990;5:826–829
3. Grifo JA, Tang YX, Cohen J, Sanyal MK, Rozenkwaks Z: Ongoing pregnancy in a hemophilia carrier by embryo biopsy and simultaneous amplification of X and Y chromosome specific DNA from a single blastomere. JAMA 1992; 6:727–729
4. Handyside AH, Lesko JG, Tarin JJ, Winston RML, Hughes MR: Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis. N Engl J Med 1992;327(13):905–909
5. Verlinsky Y, Handyside A, Simpson JL, Edwards R, Kuliev A, Muggleton-Harris A, Readhead C, Liebaers I, Coonen E, Plachot M, Carson S, Strom C, Braude P, Van Steirteghem A, Monk M, Ginsberg N, Pieters M, De Sutter P, Gimenez C, Kontogianni E, Matthews C, Wilton L: Current progress in preimplantation genetic diagnosis. J Assist Reprod Genet 1993;10(5):353–360
6. Harper J, Handyside AH: The current status of preimplantation diagnosis. Curr Obstet Gynecol 1994;4:143–149
7. Handyside AH, Delhanty JDA: Cleavage stage biopsy of human embryos and diagnosis of X-linked recessive disease. *In* Preconception and Preimplantation Diagnosis of Human Genetic Disease, RG Edwards (ed). Cambridge, Cambridge University Press, 1993, pp 239–270
8. Verlinsky Y, Kuliev AM: Preimplantation diagnosis of Genetic Diseases: A New Technique in Assisted Reproduction. New York, Wiley–Liss, 1993
9. Grifo JA, Tank YX, Munné S, Alikani M, Cohen J, Rosenwaks Z: Healthy deliveries from biopsied human embryos. Hum Reprod 1994;9(5):912–916
10. Griffin DK, Wilton LJ, Handyside AH, Winston RML: Diagnosis of sex in preimplantation embryos by fluorescent in situ hybridization. Hum Genet 1992;89:18–22
11. Munné S, Weier HUG, Stein J, Grifo J, Cohen J: A fast and efficient method for simultaneous X and Y in situ hybridization of human blastomeres. J Assist Reprod Genet 1993;10: 82–90
12. Delhanty JDA, Griffin DK, Handyside AH, Harper J, Atkinson GHG, Pieters MHEC, Winston RML: Detection of aneuploidy and chromosomal mosaicism in human embryos during pre-

implantation sex determination by fluorescent in situ hybridisation (FISH). Hum Mol Genet 1993;2(8):1183–1185
13. Munné S, Grifo J, Cohen J, Weier HUG: Chromosome abnormalities in Human Arrested preimplantation embryos: A multiple-probe FISH study. Am J Hum Genet 1994;55:150–159
14. Chong SS, Kristjansson K, Cota J, Handyside AH, Hughes MR: Preimplantation prevention of X-linked disease: Reliable and rapid sex determination of single human cells by restriction analysis of simultaneously amplified ZFX and ZFY sequences. Hum Mol Genet 1993;2(8):1187–1191
15. Levinson GL, Fields RA, Harton GL, Palmor FT, Maddalena A, Fugger EF, Schulman JD: Reliable gender screening for human preimplantation embryos, using multiple DNA target-sequences. Hum Reprod 1992;7:1304–1313
16. Liu J, Lissens W, Devroy P, Van Steirteghem A, Liebaers I: Amplification of X- and Y-chromosome-specific regions from single human blastomeres by polymerase chain reaction for sexing of preimplantation embryos. Hum Reprod 1994;9: 716–720
17. Munné S, Weier HUG, Grifo J, Cohen J: Chromosome mosaicism in human embryos. Biol Reprod 1994;51:373–379
18. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J: Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod 1993;8(12):2185–2191
19. Munné S, Tang YX, Grifo J, Rosenwaks Z, Cohen J: Sex determination of human embryos using polymerase chain reaction and confirmation by fluorescence in situ hybridization. Fertil Steril 1994;61:111–117
20. Liu J, Lissens W, Devroey P, Van Steirteghem A, Liebaers I: Polymerase chain reaction analysis of the cystic fibrosis ΔF508 mutation in human blastomeres following oocyte injection of a single sperm from a carrier. Prenatal Diagn 1993; 13:878–880
21. Verlinsky Y, Milayeva S, Evsikov S, *et al.*: Preconception and preimplantation diagnosis for cystic fibrosis. Prenatal Diagn 1992;12:103–110
22. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N: Whole genome amplification from a single cell: Implications for genetic analysis. Proc Natl Acad Sci USA 1992;89:5847–5851
23. Kristjansson K, Chong SS, Van den Veyver IG, Subramanian S, Snabes MC, Hughes MR: Preimplantation single cell analyses of dystrophin gene deletions using whole genome amplification. Nature Genet 1994;6:19–23
24. Tully G, Sullivan KM, Gill P: Analysis of 6 VNTR loci by "multiplex" PCR and automated fluorescent detection. Hum Genet 1993;92:554–562
25. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D: Comparitive genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992;258:818–821
26. Thornhill A, Holding C, Monk M: Recycling the single cell to detect specific chromosomes and to investigate specific gene sequences. Hum Reprod (in press)
27. Verlinsky Y, Morozov G, Gindilis V, Freidine M, Rechitsky S, Verlinsky O, Ivakhnenko V, Zdanovsky V, Strom CM, Kuliev A: Homeobox gene expression in human oocytes and preembryos. Mol Reprod Dev (in press)
28. Verlinsky Y, Dozortsev D, Evsikov S: Visualization and cytogenetic analysis of second polar body chromosomes following its fusion with one-cell mouse embryo. J Assist Reprod Genet 1994;11:123–131
29. Dyban A, De Sutter P, Verlinsky Y: Okadaic acid induces premature chromosome condensation reflecting the cell cycle progression in one cell stage mouse embryos. Mol Reprod Dev 1993;34(4):403–415
30. Griffin DK, Handyside AH, Harper JC, Wilton LJ, Atkinson GHG, Soossis L, Wells D, Kontogianni EH, Tarin J, Geber S, Ao A, Winston RML, Delhanty JDA: Clinical experience with preimplantation diagnosis of sex by dual fluorescent in situ hybridisation. J Assist Reprod Genet 1994;11:132–143
31. Harper JC, Coonen E, Handyside AH, Winston RML, Hopman AHN, Delhanty JDA: Mosaicism of autosomes and sex chromosomes in morphologically normal monospermic preimplantation human embryos. Prenatal Diagn (in press)

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