

# Double locus analysis of chromosome 21 for preimplantation genetic diagnosis of aneuploidy

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Preimplantation genetic diagnosis (PGD) of numerical chromosome abnormalities significantly reduces spontaneous abortions and may increase pregnancy rates in women of advanced maternal age undergoing *in vitro* fertilization. However, the technique has an error rate of around 10% and trisomy 21 conceptions have occurred after PGD. To further reduce the risk of transferring trisomy 21 embryos to the patient, we designed a protocol that analyzes chromosome 21 twice by targeting two different loci. This protocol was applied to 388 embryos from 60 cycles of PGD of aneuploidy. The scoring criterion used was based on giving equal importance to both probe results. Of the 242 embryos diagnosed as abnormal, 125 were re-biopsied to assess the rate of false positives and false negatives of the protocol and their clinical relevance.

The results of the present study showed no reduction in the overall fluorescent *in situ* hybridization (FISH) error rate for single cells. However, by using a different scoring criterion, the incidence of false negative can be reduced to 1.6% without missing any trisomy 21. In addition, the present study suggests that if two or more loci from the same chromosome could be simultaneously analyzed in single cells, errors caused by false monosomies could be reduced. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: PGD; trisomy 21; monosomy 21; IVF; mosaicism

## INTRODUCTION

There are three primary objectives of preimplantation genetic diagnosis (PGD) of numerical chromosome abnormalities.

The first objective is to improve implantation rates by selecting chromosomally normal embryos for transfer, assuming that chromosomally abnormal embryos have lower potential implantation (Munné *et al.*, 1993). Based on oocyte donation data, it is now clear that the implantation decline observed with increasing maternal age is mostly due to the oocyte and not the uterus (Navot *et al.*, 1994). The most obvious link between maternal age and embryonic competence is the increase of aneuploidy with maternal age described in oocytes, embryos and spontaneous abortions (Hassold *et al.*, 1980; Warburton *et al.*, 1980; Munné *et al.*, 1995; Dailey *et al.*, 1996), as well as an increase with maternal age of mitochondrial mutations (Barritt *et al.*, 2000) and anomalies in the meiotic spindle (Battaglia *et al.*, 1996) in oocytes. The rate of chromosomal abnormalities in embryos is much higher than the rate reported in spontaneous abortions, suggesting that a sizable part of chromosomally abnormal embryos are naturally eliminated before prenatal diagnosis can be performed (reviewed by Munné and Cohen, 1998). Such embryo loss could account for the decline in implantation with maternal age; indeed, a recent study has reported increased

implantation rates after PGD when analyzing at least eight chromosome pairs (Gianaroli *et al.*, 1999a).

The second objective of PGD for numerical abnormalities is to reduce spontaneous abortions. Women aged 40 years and older spontaneously abort more than 40% of their pregnancies (Hull *et al.*, 1996). Of the chromosome abnormalities that do implant, many will spontaneously abort, and therefore PGD also decreases spontaneous abortions, as demonstrated in a previous study (Munné *et al.*, 1999).

The third objective is to reduce the chance of conceiving a trisomic baby. PGD using fluorescence *in situ* hybridization (FISH) has an overall error rate around 10% when applied to single blastomeres (Munné and Weier, 1996; Munné *et al.*, 1998a). However, only 5–6% of FISH false positive and false negatives could have a clinical relevance by either reducing the number of transferable embryos (false positive) or transferring aneuploidy embryos able to implant (false negatives) (Munné *et al.*, 1998b; Gianaroli *et al.*, 1999a). The frequency of trisomies after PGD has thereby been reduced from an expected 2.6% to 0.3% in a population of PGD patients aged 39 years or older (Munné, 2000). In fact trisomy 21 conceptions have occurred after PGD (Munné *et al.*, 1998a; International Working Group on Preimplantation Genetics, 2001). Because the FISH error rate is caused mostly by chromosome overlaps and mosaicism (Munné, 2000), a further reduction in trisomic offspring could be achieved by analyzing two cells per embryo. However, preliminary data from several laboratories indicate a significant reduction in

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implantation after the biopsy of two cells per embryo, thus opposing the first objective mentioned above (Munné, unpublished data).

The aim of the present study was to improve current FISH protocols by reducing misdiagnoses due to chromosome overlaps. Single cells were hybridized first with a cocktail of probes including one for chromosome 21, then reanalyzed with another combination of probes, including a second probe for chromosome 21 that targeted a different locus. Thus, if the loci of chromosome 21 targeted in the first hybridization overlapped, they would most probably not overlap for the second probe, showing a different result. Two probes have been previously used on the same chromosome to determine chromosome translocations and gonosomal mosaicism (Munné *et al.*, 1998c, 2000; Conn *et al.*, 1999). In the present study they were used to predict aneuploidy of chromosome 21 without a previous genetic condition.

The present study was designed with the finality of keeping to a minimum the risk of diagnosing false negatives for chromosome 21 in view of the clinical relevance associated with trisomy 21.

## PATIENTS AND METHODS

### PGD cases

Patients undergoing PGD of aneuploidy were either women of advanced maternal age, who had a history of repeated spontaneous abortion, or women that had repeated IVF failure. On Day 3 of development, each embryo had only one cell biopsied (Grifo *et al.*, 1992), unless the cell did not produce a nucleus after fixation, in which case a second cell was biopsied. Fixation was performed as described previously (Munné *et al.*, 1996). Normally developing embryos classified as chromosomally normal by PGD were transferred to the patient on Day 4 (Gianaroli *et al.*, 1999b). Some non-transferred embryos were reanalyzed with all or most of their cells fixed individually as described previously (Munné *et al.*, 1996). Not all non-transferred embryos were reanalyzed either because of time constraints, embryo degeneration in culture, or because the abnormality that they carried was not interesting for the study. For instance, chaotic embryos, with most cells having a different abnormal chromosome complement, are not useful for determining FISH efficiencies and therefore were not analyzed.

Embryos were analyzed either at The Institute for Reproductive Medicine and Science at Saint Barnabas Medical Center (Livingston, NJ, USA), or at S.I.S.Me.R. (Bologna, Italy), in accordance with guidelines approved by the respective internal review boards including written consent from each patient.

### FISH procedure

Fixed cells were analyzed by two rounds of FISH. The first hybridization used probes for chromosomes 16 (CEP<sup>™</sup> 16 satellite II, D16Z3, 16q11.2) and 18

(CEP 18, alpha satellite, D18z1, 18p11.1-q11.1) and locus-specific probes for chromosomes 13 (RB1 locus gene within 13q14), 21 (locus D21S259, D21S341 and D21S342 contained within the 21q22.13 to 21q22.2 region), and 22 (BCR gene locus). The second round of hybridization included satellite probes for chromosomes X (CEP X alpha satellite, Xp11.1-q11.1), Y (CEP Y alpha satellite, Yp11.1-q11.1) and 15 (CEP 15 satellite III, 15q11.2) and a subtelomeric probe for chromosome 21 (VIJyRM2029 locus). The first hybridization used the MultiVysion<sup>™</sup> PB multicolor probe panel hybridization mixture commercialized by Vysis (Downers Grove, IL, USA) with the probe for chromosomes 13 labeled in SpectrumRed<sup>™</sup>, 16 with SpectrumAqua<sup>™</sup>, 18 with SpectrumBlue<sup>™</sup>, 21 with SpectrumGreen<sup>™</sup> and 22 with SpectrumGold<sup>™</sup>. The second hybridization mixture was prepared at Vysis, but was not commercially available at the time. It consisted of probes for chromosomes X, Y, 15, and 21 labeled respectively in SpectrumAqua<sup>™</sup>, SpectrumGold<sup>™</sup>, SpectrumGreen<sup>™</sup> and SpectrumRed<sup>™</sup>, and ranging in concentration between 25 and 100 ng/μl.

Aliquots (10 μl) of the first hybridization solution were applied to the glass slide containing fixed blastomeres and covered with a 18 × 18 mm coverslip. The slide was then placed for 5 min on a slide warmer preheated to 73°C, sealed with rubber cement, and placed in a dark moist chamber at 37°C for 3 h. After the hybridization, the slides were washed individually at 71°C in 0.7 × SSC for 4 min. The slides were then mounted with 10 μl antifade solution and observed with a fluorescent microscope (Olympus BX70) equipped with single-band pass filters for each fluorochrome used. Images were composed with the ISIS v3 Metasystem software.

After the analysis of the first set of probes, the slides were washed in 0.7 × SSC at room temperature until the coverslips fell off, dipped in distilled water at 71°C for 10 s, and then dehydrated (70%, 85%, 100% ethanol, 2 min each). Aliquots (10 μl) of the second hybridization solution were applied per slide and the slide was processed in the same way as for the first hybridization with the difference that it was washed for only 30 s in 0.7 × SSC at 71°C. The washed slides were then mounted with DAPI in antifade (Vysis) and analyzed.

The criterion used for scoring FISH signals of chromosomes analyzed with a single probe was as previously described (Munné and Weier, 1996) with the exception of the scoring for chromosome 21, which was as follows. (1) If one of the two 21 probes showed one signal and the other had two, the diagnosis was disomy 21 (normal). (2) If one of the probes showed unclear results indicating either one or two signals, and the other probe had two signals, the result was disomy 21. (3) If one of the probes showed two signals and the other had three, the result was trisomy 21. (4) If one of the probes showed unclear results indicating either two or three signals, and the other probe had two signals, the result was trisomy 21. (5) If one of the probes showed unclear results indicating either two or three signals, and the other probe had three signals, the result was trisomy 21.

To classify chromosome abnormality types by FISH, the same criteria described by Munné *et al.* (1998b) were used. However, during the reanalysis of whole embryos a small change was made whereby mosaics with less than 38% abnormal cells were considered normal and the abnormal cells were considered FISH errors because it was difficult to differentiate mosaics from FISH errors in embryos with six cells or less.

## RESULTS

A total of 388 embryos from 60 cycles were analyzed by PGD following the above mentioned criteria. Of those, 382 were diagnosed: 140 were classified as normal, 132 aneuploid, 71 chaotic, 20 haploid and 19 polyploid. Of the non-transferred embryos, 125 were analyzed by FISH using the same protocol used for PGD. As indicated in Table 1, 17 (13.6%) were misdiagnosed, including 13 classified as trisomic when in fact they were normal for that chromosome, one classified as normal that was in fact monosomic, another classified as normal that was trisomic, and two embryos classified one as a single monosomy and the other as a double monosomy that were normal. Eight of the misdiagnoses were caused by false trisomy 21 (Table 1). Therefore, the incidence of false positives and false negatives was 12% and 1.6%, respectively.

Table 2 shows the different combinations of results for each chromosome 21 probe and the errors produced. Examples of unclear LSI21 results and the respective Tel21q results are shown in Figures 1–4. Most embryos diagnosed as normal, monosomies and trisomies were confirmed by reanalysis when both probes had the same result, but when the results were different, they caused eight false diagnoses of trisomy 21. However, if other criteria for diagnosing chromosome 21 had been used, the error rate could have been reduced (Table 3). Of the five possibilities of scoring the results for the two 21 probes (Table 3), criterion 5

Table 2—PGD and reanalysis results for probes LSI21 and Tel 21q, and diagnosis according to the scoring criteria used

LSI21	Tel21q	Analyzed embryos	PGD	Confirmed embryos	Type error
1	1	44	Monosomy	14/14	0
1 or 2	1	1	Monosomy	1/1	0
1 or 2	2	4	Normal	3/3	0
1	2	4	Normal	2/2	0
2	1	4	Normal	2/2	0
2	2	268	Normal	79/79	0
0	0	7	Null	0	0
2	3	8	Trisomy	0/4	4
					Normal
3	3	22	Trisomy	10/10	0
2	2 or 3	1	Trisomy	0/1	1
					Normal
2 or 3	3	3	Trisomy	2/2	0
2 or 3	2 or 3	2	Trisomy	0/2	2
					Normal
2 or 3	NR	2	Trisomy	1/2	1
					Normal
4	4	18	Polyploid	4/4	0

NR, no result.

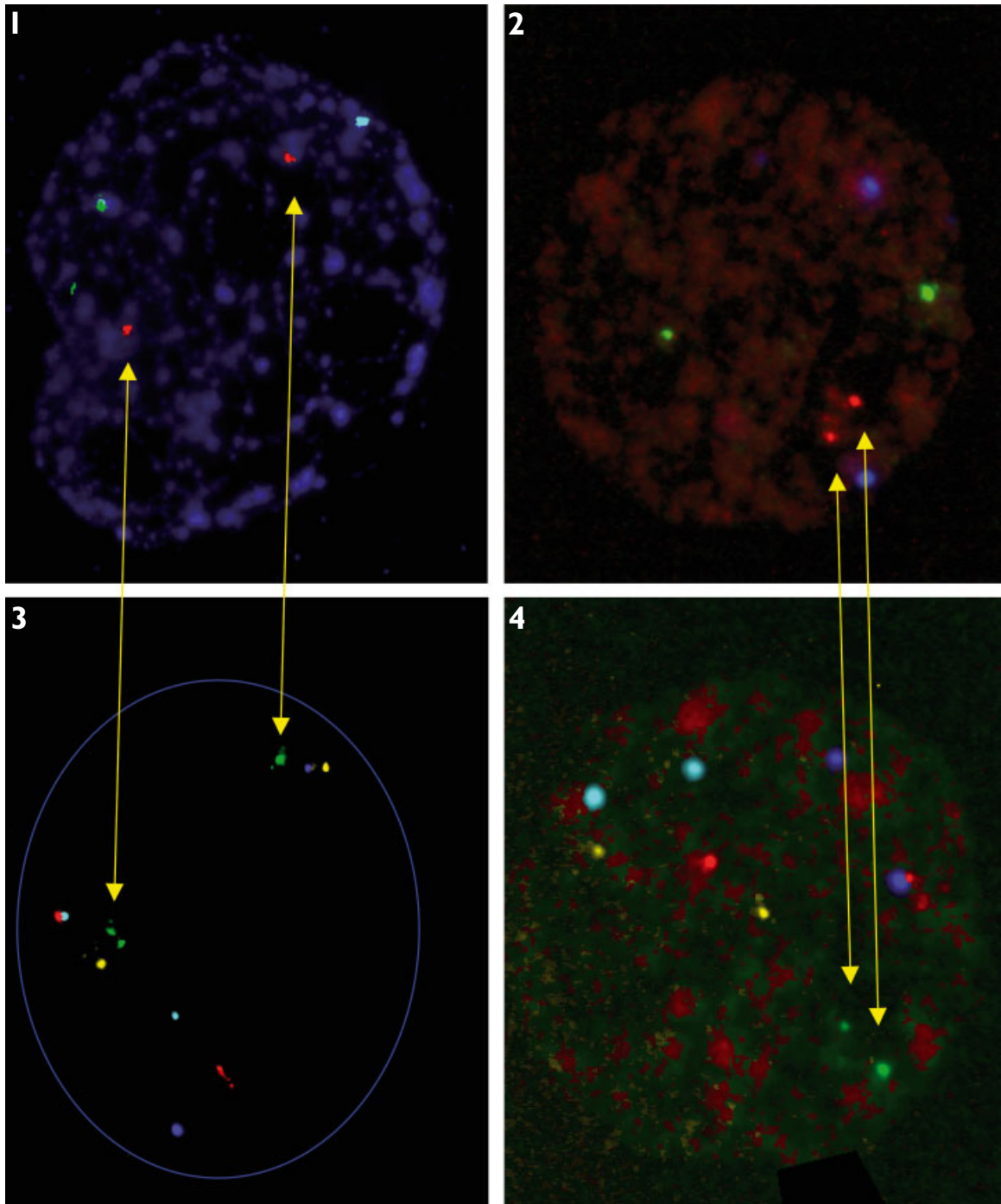
could have produced the highest reliability with no misdiagnosed trisomy 21, by using Tel21q to confirm monosomies, and considering any dubious LSI-21 results, that could have been either two or three signals, as three signals. The other criteria would have increased either the total rate of inaccuracy or the number of missed trisomies (Table 3). If criterion 5 had been used, the risk of transferring a false negative embryo would have been 2.4%.

## DISCUSSION

There are several sources of FISH error in PGD, namely the loss of micronuclei during fixation, signal overlaps and mosaicism. Our fixation method prevents

Table 1—Embryos misdiagnosed after PGD

Cycle	Embryo	PGD result	Cells analyzed	Reanalysis
21073	13	Monosomy 13 and 22	3	Normal
200252	5	Monosomy 18	5	Normal
22032	2	Normal	12	Trisomy 22
200254	1	Normal	5	Monosomy 22
21189	4	Trisomy 13	5	Normal
200265	7	Trisomy 18	4	Normal
200266	4	Trisomy 21	6	Mosaic for 22 (71%)
200252	3	Trisomy 21	7	Normal
200271	9	Trisomy 21	8	Normal
200263	4	Trisomy 21	9	Normal
200239	4	Trisomy 21	10	Normal
200281	5	Trisomy 21	12	Normal
200282	2	Trisomy 21, monosomy 15	7	Monosomy 15
200287	2	Trisomy 21 and 16	11	Normal
200282	3	Trisomy 22	7	Normal
200271	6	Trisomy XXX	8	Normal
200239	7	Trisomy XXY	14	Normal



Figures 1 (top left) and 3 (bottom left) show blastomeres A and B hybridized with probes for chromosomes 13 (red), 16 (blue), 18 (aqua), LSI21 (green) and 22 (yellow). Figure 2 (top right) and 4 (bottom right) show blastomeres A and B rehybridized with probes for chromosomes X (green), Y (yellow), Tel21q (red) and 15 (blue or aqua). In the first hybridization, one of the LSI21 signals of blastomere A (Figure 1) was split but in the second hybridization the Tel21q shows only two clear signals (Figure 2). The first hybridization of blastomere B (Figure 3) showed one signal of LSI21 much smaller than the other one, however, after the second hybridization (Figure 4) both Tel21q signals were similar. Blastomere A was in addition monosomic for chromosome 15

the loss of micronuclei (Munné *et al.*, 1998b), while overlaps can be minimized but not completely eliminated using a good spreading method that produces large fixed nuclei (Munné *et al.*, 1996). To further minimize FISH errors produced by overlaps we expected that if two copies of the same chromosome are overlapping at the specific locus targeted by one

DNA probe, the same chromosomes would not overlap at another locus. Thus, specific targeting of a different second locus should have improved the accuracy of the diagnosis.

However, the present results indicate that when the first panel of probes did not produce a clear signal, or when there were discordances between the first and

Table 3—Misdiagnosed embryos for chromosome 21 depending on the scoring criteria used

Criteria	Percentage misdiagnosed ( <i>n</i> )		
	Normal	Trisomic	Monosomic
1. Used in this study	6.3 (8)	0	0 (0)
2. Tel21 not used, 2 or 3 LSI21 as 2	2.4 (3)	2.4 (3)	1.6 (2)
3. Tel21 not used, 2 or 3 LSI21 as 3	2.4 (3)	0	1.6 (2)
4. Tel21 for monosomies, 2 or 3 LSI21 as 2	2.4 (3)	2.4 (3)	0 (0)
5. Tel21 for monosomies, 2 or 3 LSI21 as 3	2.4 (3)	0	0 (0)

second panel of probes, inaccurate diagnoses were still produced. Most probably the cause of such errors is that locus-specific probes, such LSI21 and Tel21q, are less efficient in second and third cell rehybridizations of single cells. It remains to be determined if the cause of the poor performance of Tel21q in rehybridizations is due to the quality of the DNA, damaged by two denaturations and washes, or by the combination of locus-specific and satellite probes in the hybridization mixture. Evidence from PGD of translocation cases, in which telomeric probes are used alone for rehybridizing single cells, indicates better and more accurate results (Munné *et al.*, 2000). However, using Tel21q alone in the second panel would have prevented analysis of chromosomes 15, X and Y, unless a third hybridization step were to be performed.

Nevertheless, the present results indicate that this cocktail of probes can still be used to reduce the misdiagnosis of normal embryos as monosomies. Although the criterion used was not appropriate to differentiate between disomy and trisomy 21 when there were discrepancies between the two 21 probes, a different criterion could have prevented some misdiagnoses. As shown in Table 3, the best criterion to prevent misdiagnosis of aneuploidy 21 (criterion 5) was when Tel21q is considered only when the LSI21 reading is 1, or between 1 and 2, i.e. when an overlap may have occurred. In that case, fewer normal embryos are misdiagnosed as monosomies. By the same criterion, when the LSI21 reading is not clear, or between 2 and 3, Tel21q should not be considered; and the LSI21 should be considered as having three signals and the embryo not transferred. If criterion 5 had been used, the risk of transferring false negative embryos would have been 1.6%. Although criterion 5 would misclassify 2.4% of normal embryos as trisomic, it would not miss any trisomy, and for the purpose of preventing trisomic offspring it is better to ascertain more trisomies even at the expense of discarding a few potentially normal embryos.

All the embryos diagnosed as normal by PGD were confirmed to be euploid for the chromosomes studied following reanalysis (86/86). This confirms the validity of the adopted scoring criteria in the case of two clearly visible signals per chromosome.

In conclusion, the double locus analysis for chromosome 21 presented here permitted the detection of all the trisomy 21 embryos screened. This was due to a very careful attitude in the diagnosis of trisomies, especially trisomy 21, whose clinical relevance is critical. Conversely, the retrospective analysis of the scoring criteria implemented in the present study allowed the identification of those associated with the highest reliability in combination with the best safeguard for the patient.

Based on the present results, rehybridizing cells with telomeric probes is valuable for rescuing monosomies due to failed hybridization. For this purpose, telomeric probes could be used, probably not mixed with satellite probes, for confirmation of monosomies. This reanalysis could be done on a third rehybridization, although the efficiency of a third-round hybridization remains to be determined. Other studies analyzing the results of three hybridizations on the same cell have not measured the rate of false monosomies and trisomies, only the presence of signals (Liu *et al.*, 1998). A third hybridization could improve diagnosis of trisomy, analyzing for two or even three loci on the same chromosome, as long as the probes work with high efficiency. As a general consideration, the clinical relevance of chromosome 21 is so high that the use of two different probes cannot be disregarded as a safety measure irrespective of the risk of misdiagnosing some euploid embryos.

Hopefully in the near future the use of comparative genome hybridization (CGH) and related techniques (Kallioniemi *et al.*, 1992; Wells and Delhanty, 2000) will allow the simultaneous analysis of multiple loci on the same hybridization, permitting a more robust analysis of single cells.

## REFERENCES

- Barritt JA, Cohen J, Brenner CA. 2000. Mitochondrial point mutation in human oocytes is associated with maternal age. *Reprod Biomed Online* 1: 96–100.
- Battaglia DE, Goodwin P, Klein NA, Soules MR. 1996. Influence of maternal age on meiotic spindle in oocytes from naturally cycling women. *Hum Reprod* 11: 2217–2222.
- Conn CM, Cozzi J, Harper JC, Winston RML, Delhanty JDA. 1999. Preimplantation genetic diagnosis for couples at high risk of Down syndrome pregnancy due to parental translocation or mosaicism. *J Med Genet* 36: 45–50.
- Dailey T, Dale B, Cohen J, Munné S. 1996. Association between non-disjunction and maternal age in meiosis-II human oocytes detected by FISH analysis. *Am J Hum Genet* 59: 176–184.
- Gianaroli L, Magli MC, Ferraretti AP, Munné S. 1999a. Preimplantation diagnosis for aneuploidies in patients undergoing *in vitro* fertilization with a poor prognosis: identification of the categories for which it should be proposed. *Fertil Steril* 72: 837–844.
- Gianaroli L, Magli MC, Munné S, Fortini D, Ferraretti AP. 1999b. Advantages of day 4 embryo transfer in patients undergoing preimplantation genetic diagnosis of aneuploidy. *J Assist Reprod Genet* 16: 170–175.
- Grifo JA, Tang YX, Cohen J, Gilbert F, Sanyal MK, Rosenwaks Z. 1992. Ongoing pregnancy in a hemophilia carrier by embryo biopsy and simultaneous amplification of X and Y chromosome specific DNA from single blastomeres. *JAMA* 267: 727–729.
- Hassold T, Chen N, Funkhouser J, *et al.* 1980. A cytogenetic

- study of 1000 spontaneous abortuses. *Ann Hum Genet Lond* **44**: 151–178.
- Hull MGR, Fleming CF, Hughes AO, McDermott A. 1996. The age-related decline in female fecundity: a quantitative controlled study of implanting capacity and survival of individual embryos after *in vitro* fertilization. *Fertil Steril* **65**: 783–790.
- International Working Group on Preimplantation Genetics. 2001. 10th Anniversary of Preimplantation Genetic Diagnosis. *J Assist Reprod Genet* **18**: 64–70.
- Kallioniemi A, Kallioniemi OP, Sudar D, *et al.* 1992. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* **258**: 818–821.
- Liu J, Tsai Y-L, Zheng X-Z, Baramki TA, Yazigi RA, Katz E. 1998. Potential use of repeated fluorescence *in situ* hybridization in the same human blastomeres for preimplantation genetic diagnosis. *Fertil Steril* **70**: 729–733.
- Munné S. 2000. Preimplantation genetic diagnosis of numerical abnormalities using FISH. In *Handbook of In Vitro Fertilization* (2nd edn), Trounson AO, Gardner DK (eds). CRC Press: Boca Raton, FL; 307–326.
- Munné S, Cohen J. 1998. Chromosome abnormalities in human embryos. *Hum Reprod Update* **4**: 842–855.
- Munné S, Weier U. 1996. Simultaneous enumeration of chromosomes 13, 18, 21, X and Y in interphase cells for preimplantation genetic diagnosis of aneuploidy. *Cytogenet Cell Genet* **75**: 263–270.
- Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. 1993. diagnosis of major chromosome aneuploidies in human preimplantation embryos. *Hum Reprod* **8**: 2185–2191.
- Munné S, Alikani M, Tomkin G, Grifo J, Cohen J. 1995. Embryo morphology, developmental rates and maternal age are correlated with chromosome abnormalities. *Fertil Steril* **64**: 382–391.
- Munné S, Dailey T, Finkelstein M, Weier HUG. 1996. Reduction in signal overlap results in increased FISH efficiency: implications for preimplantation genetic diagnosis. *J Assist Reprod Genet* **13**: 149–156.
- Munné S, Magli C, Bahçe M, *et al.* 1998a. Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. *Prenat Diagn* **18**: 1459–1466.
- Munné S, Márquez C, Magli C, Morton P, Morrison L. 1998b. Scoring criteria for preimplantation genetic diagnosis of numerical abnormalities for chromosomes X, Y, 13, 16, 18 and 21. *Mol Hum Reprod* **4**: 863–870.
- Munné S, Fung J, Cassel MJ, Márquez C, Weier HUG. 1998c. Preimplantation genetic analysis of translocations: case-specific probes for interphase cell analysis. *Hum Genet* **102**: 663–674.
- Munné S, Magli C, Cohen J, *et al.* 1999. Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. *Hum Reprod* **14**: 2191–2199.
- Munné S, Sandalinas M, Escudero T, Fung J, Gianaroli L, Cohen J. 2000. Outcome of preimplantation genetic diagnosis of translocations. *Fertil Steril* **73**: 1209–1218.
- Navot D, Drews MR, Bergh PA, *et al.* 1994. Age related decline in female fertility is not due to diminished capacity of the uterus to sustain embryo implantation. *Fertil Steril* **61**: 97–101.
- Warburton D, Stein Z, Kline J, Susser M. 1980. Chromosome abnormalities in spontaneous abortion: data from the New York City study. In *Human Embryonic and Fetal Death*, Porter LH, Hook EB (eds). Academic Press: New York, NY; 261–287.
- Wells D, Delhanty JDA. 2000. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod* **6**: 1055–1062.