

# Chromosomal abnormalities and embryo development in recurrent miscarriage couples

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**BACKGROUND:** Chromosomal abnormalities are an important cause of spontaneous abortion and recurrent miscarriage (RM). Therefore, we have analysed the incidence of chromosomal abnormalities and embryo development in patients with RM. **METHODS:** Preimplantation genetic diagnosis (PGD) was performed on 71 couples with RM and 28 couples undergoing PGD for sex-linked diseases (control group). Chromosomes 13, 16, 18, 21, 22, X and Y were analysed by fluorescence in-situ hybridization. **RESULTS:** The implantation rate in RM patients was 28% and three patients (13%) miscarried. The percentage of abnormal embryos was significantly increased ( $P < 0.0001$ ) in RM patients compared with controls (70.7 versus 45.1%). All of the embryos were abnormal in 19 cycles (22.1%) and repeated PGD cycles yielded similar rates of chromosomal abnormalities in 14 couples. Anomalies for chromosomes 16 and 22 were significantly higher ( $P < 0.01$ ) in RM cases. In the RM population, euploid embryos reached the blastocyst stage more frequently than abnormal embryos (61.7 versus 24.9%;  $P < 0.0001$ ). **CONCLUSIONS:** RM is associated with a higher incidence of chromosomally abnormal embryos, of which some are able to develop to the blastocyst stage. IVF plus PGD is an important step in the management of these couples, but the technique has to move towards a full chromosome analysis.

*Key words:* aneuploidy/blastocyst/FISH/PGD/recurrent miscarriage

## Introduction

Recent advances in reproductive medicine and molecular cytogenetics have changed the approach to the infertile couple. The introduction of fluorescence in-situ hybridization (FISH) has enabled the chromosomal assessment of embryos. The initial applications of preimplantation genetic diagnosis (PGD) to prevent X-linked recessive diseases (Griffin *et al.*, 1991; Veiga *et al.*, 1994; Vandervorst *et al.*, 2000) and the unbalanced transmission of parental balanced translocations (Conn *et al.*, 1998; Munné *et al.*, 2000; Scriven *et al.*, 2001) have widened to improve IVF results in repetitive implantation failure, increased maternal age (Gianaroli *et al.*, 1999; Munné *et al.*, 1999; Kahraman *et al.*, 2000) and recurrent miscarriage (RM) patients (Simón *et al.*, 1998; Vidal *et al.*, 1998; Pellicer *et al.*, 1999).

We introduced PGD in the reproductive treatment of RM couples for two reasons. Firstly, because even after an appropriate infertility work-up, almost 50% of cases remain classified as unknown aetiology (Coulam, 1986; Clifford *et al.*, 1994). Secondly, because it is well documented that chromosomal abnormalities are involved in first trimester spontaneous abortions. Cytogenetic evaluations of these specimens have

revealed an overall incidence of chromosomal abnormalities of 50–70% (Boué *et al.*, 1975; Hassold *et al.*, 1978; Plachot, 1989; Eiben *et al.*, 1990; Stephenson *et al.*, 2002). Only 4.7% of couples with two or more abortions include a carrier of a balanced structural abnormality (De Braekeleer and Dao, 1990). The most common cause of spontaneous abortions is de novo numerical abnormalities, in particular autosomal trisomies for chromosomes 13, 14, 15, 16, 21 and 22, followed by monosomy X (Hassold *et al.*, 1980; Strom *et al.*, 1992; Stephenson *et al.*, 2002).

In nature, the incidence of chromosomal abnormalities decreases over the duration of pregnancy in such a manner that in stillborns it is ~6% (Machín and Crolla, 1974) and in live births 0.6% (Nielsen, 1975), as has been shown for the most common trisomies (Jacobs and Hassold, 1995). This pattern of negative selection against chromosomal abnormalities between implantation and birth operates during the pre-implantation period. In fact, autosomal monosomies are rarely found in spontaneous abortions and are thought to be responsible for preclinical abortions (Boué *et al.*, 1975; Hassold *et al.*, 1980; Stephenson *et al.*, 2002). This mechanism of natural selection may also operate during preimplantation embryogenesis, with

a progressive loss of abnormal embryos at specific stages in early development, through developmental arrest and degeneration of abnormal embryos. By employing IVF and PGD, we are able to observe *in vitro* the developmental ability of human embryos at these stages; we can learn about their behaviour, and perhaps about the mechanisms involved in the genetic causes of RM.

With these objectives, in 1996 we started a PGD programme in RM patients in which euploid embryos were transferred on day 5. We included patients with two previous consecutive early miscarriages, because a high incidence of chromosomal abnormalities has been found in cytogenetic studies of spontaneous abortions in these couples (Ogasawara *et al.*, 2000). In the first series of nine cycles analysed, we showed an increased rate of chromosomal abnormalities in embryos derived from patients with RM as compared with controls (Simón *et al.*, 1998; Vidal *et al.*, 1998; Pellicer *et al.*, 1999). We have continued this work in a prospective manner to confirm the results in a larger series and to find out the diagnostic and/or therapeutic advantages of PGD in this population. Additionally, we describe the incidence of chromosomal abnormalities found in these embryos, and their developmental ability.

## Materials and methods

### Patients and IVF procedure

The PGD programme for RM patients was approved by the institutional review board at the Instituto Valenciano de Infertilidad, and patients gave written consent before entering the study. A completely normal infertility work-up was mandatory to be admitted in this protocol, including: vaginal ultrasound scan and hysterosalpingography/hysteroscopy; basal serum FSH, LH, PRL, TSH and glucose levels; screening for thrombophilia with the measurement of plasma levels of antithrombin, proteins C and S, and antiphospholipid antibodies; and parent's karyotypes. In the RM group, couples with two or more previous abortions and a normal infertility work-up were included. The cut-off number of mature follicles was the standard for an IVF cycle in our centre: six or more mature follicles ( $\geq 15$  mm). A control group of couples undergoing PGD because of the risk of sex-linked diseases and without other infertility problems was included to compare clinical results and the incidence of abnormalities for each chromosome with the RM group. In the control group, the inclusion criteria for mature follicles were the same as the study group, as well as the number of previous IVF failures ( $0.9 \pm 1.5$  and  $0.3 \pm 0.7$  respectively) and the duration of stimulation ( $10.2 \pm 1.7$  days in the RM group and  $12.6 \pm 1.6$  in the control group).

The RM group was formed of 71 couples classified into two groups according to their age:  $<37$  years ( $n = 51$ ) and  $\geq 37$  years ( $n = 20$ ). The mean age was  $35.6 \pm 3.0$  years with a mean number of  $2.9 \pm 1.0$  previous abortions. Most of these couples were in fact infertile, and they needed IVF treatment because of salpingectomy after an ectopic pregnancy or because of previous failures of artificial insemination in cases of male factor infertility.

In the group of RM patients who were  $<37$  years old, embryo karyotyping of previous miscarriages was possible in three patients: all were trisomic. In the group  $\geq 37$  years, five karyotypes were performed: two were chromosomally abnormal, with trisomy; the other three were euploid (one male and two female karyotypes).

The control group included 28 women and was also divided in two subgroups according to age: 15 patients  $<37$  years and 13 patients  $\geq 37$  years of age. The mean age was  $35.1 \pm 4.1$  years with  $0.3 \pm 0.6$  previous miscarriages.

Only nine cycles of the RM group were included in our previous paper (Pellicer *et al.*, 1999) and the control group was also different. In the previous paper, embryos in which only chromosomes X, Y and 18 were analysed were also included. In the present paper, the control group included cycles in which at least five chromosomes were analysed.

The ovarian stimulation protocol with GnRH analogues and gonadotrophins, and the ovum retrieval procedure, have been previously described (Pellicer *et al.*, 1996). ICSI was performed to ensure high fertilization rates in these patients and to avoid the presence of sperm bound to the zona pellucida at biopsy. Fertilization was assessed 17–20 h later. Embryos were grown in 1 ml IVF/co-culture medium (CCM) (1:1; Scandinavian IVF, Göteborg, Sweden) until they reached the 8-cell stage on day 3, and were then cultured with CCM medium on a monolayer of endometrial epithelial cells prepared as previously described (Simón *et al.*, 1999). In the study group, embryo cleavage was recorded every 24 h until embryo transfer was performed on day 5. However, in the controls, transfer was performed on day 3 after embryo biopsy and assessment of the gender of the embryos.

### PGD protocol

Embryo biopsy was performed on day 3. Embryos were placed in a droplet containing  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free medium (EB-10; Scandinavian IVF) and the zona pellucida was perforated using acidified Tyrode's solution (ZD-10; Scandinavian IVF). One or two blastomeres were removed with a bevelled aspiration pipette and individually fixed with methanol:acetic acid (3:1) under an inverted microscope, using a slightly modified Tarkowsky's protocol without hypotonic pretreatment. The assessment of chromosomal abnormalities was performed by FISH.

The FISH protocol in the study group was as follows: a first round was performed using locus-specific probes for chromosomes 13 and 21; in the second round, and after signal elimination (Vidal *et al.*, 1998), a centromeric probe for chromosome 16 and a locus-specific probe for chromosome 22 were used; and finally, in the third round, triple FISH was carried out with centromeric probes for chromosomes X, Y and 18 (all probes available from Vysis Inc., Downers Grove, IL, USA). In the control group, blastomeres were initially analysed by triple FISH using X, Y and 18 chromosome-specific probes. After embryo transfer, the chromosomal analysis was completed with a second round using dual FISH for chromosomes 13 and 21. In most cases, a third hybridization round was subsequently carried out to analyse chromosomes 16 and 22. Detection washings and signal scoring were performed following the manufacturer's instructions.

Hybridization efficiency was 92% for the blastomeres analysed in the RM group and up to 95% in the control group. Hybridization efficiencies for each probe were similar in both groups, independent of the order in which the probes were used. Therefore, technical artefacts could appear equally frequently in the two groups, and would not be responsible for any increased aneuploidy rate in the RM group compared with the controls.

The percentage of abnormal embryos in each group was estimated as the number of affected embryos divided by the number of informative embryos for the probe employed.

### Statistical analysis

For statistical comparison between groups,  $\chi^2$  analysis and Fisher's exact test were used to compare pregnancy rates and percentages of

**Table I.** Clinical results of preimplantation genetic diagnosis in recurrent miscarriage patients compared with controls

	Recurrent miscarriage group			Control group		
	Age (years)		Total	Age (years)		Total
	<37	≥37		<37	≥37	
No. of patients	51	20	71	15	13	28
No. of cycles	63	23	86	18	17	35
Mean age ± SD (years)	33.2 ± 2.1	38.4 ± 1.5	35.6 ± 3.0	32.0 ± 3.0	38.5 ± 1.7	35.1 ± 4.1
Mean no. previous abortions ± SD	2.9 ± 1.0	2.4 ± 0.7	2.9 ± 1.0	0.1 ± 0.3	0.5 ± 0.8	0.3 ± 0.6
No. of transfers (%)	49 (77.8)	18 (78.3)	67 (77.9)	17 (94.4)	14 (82.3)	31 (88.6)
Mean no. embryos transferred ± SD	1.5 ± 1.0	1.4 ± 1.0	1.5 ± 1.0	2.1 ± 1.4	1.5 ± 1.1	1.8 ± 1.3
No. of pregnancies per transfer (%)	19 (38.8)	4 (22.2)	23 (34.3)	8 (47.1)	1 (7.1)	9 (29.0)
Implantation rate	30.8	19.4	28.0	32.4	3.9	20.6
No. of ectopic pregnancies (%)	0	1 (25)	1 (4.3)	0	0	0
No. of miscarriages (%)	2 (10.5)	1 (25) <sup>b</sup>	3 (13.0)	0	0	0
No. of ongoing pregnancies	7	2	9	3	1	4
No. of live births	13 <sup>a</sup>	0	13	6 <sup>a</sup>	0	6

Recurrent miscarriage group versus control group: <sup>a</sup>Ten and five pregnancies; <sup>b</sup>47,XY,+15.

**Table II.** Fluorescence in-situ hybridization results in embryos of recurrent miscarriage patients and controls

	Recurrent miscarriage group			Control group		
	Age (years)		Total	Age (years)		Total
	<37	≥37		<37	≥37	
No. of MII oocytes	918	334	1252	193	219	412
No. of 2PN	650	227	877	148	149	297
No. of biopsied embryos	487	171	658	121	115	236
No. of analysed embryos	455	150	605	112	112	224
No. of informative embryos (%)	426 (93.6)	133 (88.7)	559 (92.4)	111 (99.1)	104 (92.9)	215 (96.0)
No. of abnormal embryos (%)	301 (70.7) <sup>a</sup>	94 (70.7) <sup>b</sup>	395 (70.7) <sup>c</sup>	37 (33.3) <sup>a</sup>	60 (57.7) <sup>b</sup>	97 (45.1) <sup>c</sup>
No. of aneuploid embryos (%)	237 (55.6) <sup>d</sup>	79 (59.4)	316 (56.5) <sup>e</sup>	24 (21.6) <sup>d</sup>	49 (47.1)	73 (33.9) <sup>e</sup>
No. of haploid embryos (%)	20 (4.7)	4 (3.0)	24 (4.3)	3 (2.7)	0	3 (1.4)
No. of triploid embryos (%)	1 (0.2)	0	1 (0.2)	1 (0.9)	3 (2.9)	4 (1.9)
No. of tetraploid (%)	6 (1.4)	0	6 (1.1)	6 (5.4)	1 (1.0)	7 (3.3)

Recurrent miscarriage group versus control group: <sup>a,c,d,e</sup> $P < 0.0001$ ; <sup>b</sup> $P = 0.0406$ . MII = metaphase II; 2PN = two pronuclei.

abnormal embryos respectively. A boxplot graphic was applied to test intra-patient differences when they underwent two PGD cycles.  $P < 0.05$  was considered statistically significant. The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA).

## Results

The clinical outcome is described in Table I. In total, 86 PGD cycles were performed in 71 RM couples. In 19 of these cycles (22.1%), all embryos were diagnosed as abnormal and no transfer was performed, and in the other 67 cycles a mean number of  $1.5 \pm 1.0$  embryos were transferred, resulting in 23 pregnancies (34.3% pregnancy rate). Ten of these resulted in 13 live births. Nine are still ongoing (>20 weeks gestation), one was an ectopic pregnancy and three ended in miscarriages. All three miscarriages were carefully biopsied before removal from the uterine cavity by hysteroembryoscopy. One was a single pregnancy with a 46,XY embryo (three previous miscarriages and female age 30 years); the second was a

twin pregnancy, also with two 46,XY embryos (two previous miscarriages in five ICSI cycles, age 33 years); the third case was a 47,XY,+15 karyotype (two previous miscarriages, age 39 years).

In the control group, 35 cycles (28 couples) were included and embryo transfer was performed in 31 of them. Nine clinical pregnancies were achieved (29% pregnancy rate) with six live births and four ongoing pregnancies (>20 weeks gestation). We did not observe any statistical differences between the study group and the controls, whether they were considered as a whole ( $P = 0.6503$ ) or divided in two subgroups of age ( $P = 0.5784$ ;  $P = 0.3547$ ).

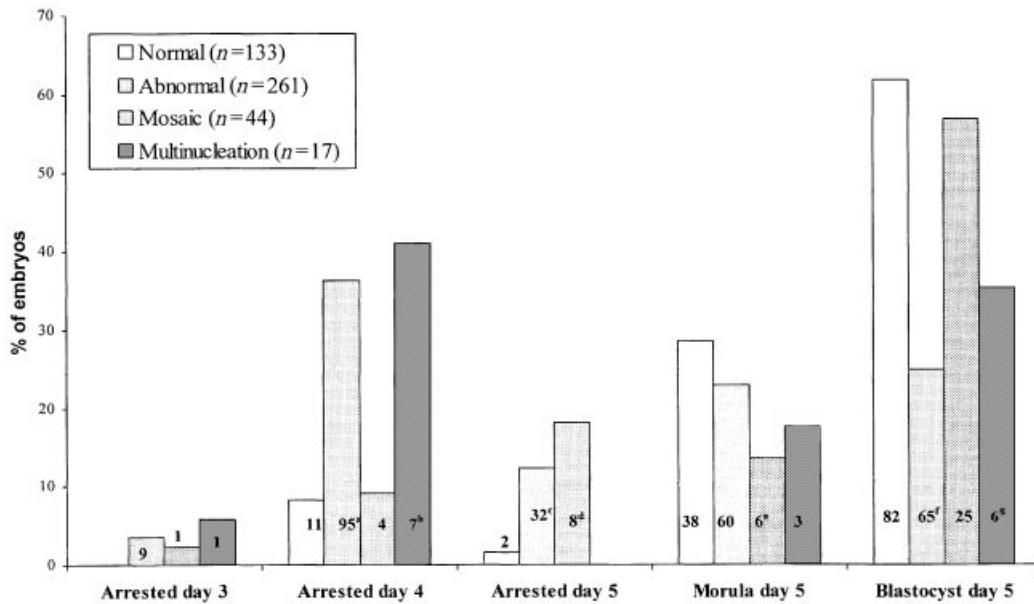
Table II shows the FISH results. A total of 559 embryos in the study group showed informative results for the chromosomes analysed, and 215 in the control group, with a significant increase in the percentage of abnormal embryos (70.7 versus 45.1%;  $P < 0.0001$ ) and in the rate of aneuploidy (56.5 versus 33.9%;  $P < 0.0001$ ) in the RM group as compared with the controls. The results were also compared separately in the two age subgroups. This showed an increased incidence in

**Table III.** Incidence of chromosomal abnormalities in recurrent miscarriage patients and controls

Chr	Recurrent miscarriage group			Control group		
	Age (years)		Total	Age (years)		Total
	<37	≥37		<37	≥37	
13 (%)	85/417 (20.4) <sup>a</sup>	25/130 (19.2)	110/547 (20.1)	6/91 (6.6) <sup>a</sup>	20/97 (20.6)	26/188 (13.8)
16 (%)	96/400 (24.0) <sup>b</sup>	33/114 (28.9)	129/514 (25.1) <sup>c</sup>	4/61 (6.6) <sup>b</sup>	7/43 (16.3)	11/104 (10.6) <sup>c</sup>
18 (%)	35/366 (9.6)	14/120 (11.7)	49/486 (10.1)	6/112 (5.4)	13/88 (14.8)	19/200 (9.5)
21 (%)	98/424 (23.1) <sup>c</sup>	37/135 (27.4)	135/559 (24.1)	8/91 (8.8) <sup>c</sup>	30/101 (29.7)	38/192 (19.8)
22 (%)	67/366 (18.3) <sup>d</sup>	25/101 (24.7)	92/467 (19.7) <sup>f</sup>	3/62 (4.8) <sup>d</sup>	6/43 (13.9)	9/105 (8.6) <sup>f</sup>
Sex chr. (%)	40/373 (10.7)	14/121 (11.6)	54/494 (10.9)	8/110 (7.3)	14/92 (15.2)	22/202 (10.9)

Recurrent miscarriage versus control group: <sup>a</sup> $P = 0.0013$ ; <sup>b</sup> $P = 0.0015$ ; <sup>c</sup> $P = 0.0052$ ; <sup>d</sup> $P = 0.0008$ ; <sup>e</sup> $P = 0.0067$ .

Chr = chromosome.



**Figure 1.** Chromosomal abnormalities and embryo development in recurrent miscarriage patients. The figures on top of the bars indicate the number of embryos analysed in each subgroup. Statistical comparisons versus normal embryos were established at each developmental stage. Comparisons versus normal embryos: <sup>a,c,f</sup> $P < 0.0001$ ; <sup>b</sup> $P = 0.001$ ; <sup>d</sup> $P = 0.0003$ ; <sup>e</sup> $P = 0.0332$ ; <sup>g</sup> $P = 0.03$

chromosomally abnormal embryos in both subgroups; however, this was more evident in younger patients ( $P < 0.0001$ ) than in older women ( $P = 0.046$ ). Aneuploidy was only increased ( $P < 0.0001$ ) in patients  $<37$  years old.

A detailed analysis of chromosomal abnormalities in the two groups (Table III) revealed significant increases in the incidence of abnormalities only for chromosomes 16 ( $P = 0.0008$ ) and 22 ( $P = 0.0067$ ) in RM patients compared with controls. However, taking age into account, there were remarkable differences: in patients  $<37$  years there was an increased incidence of abnormal embryos also for chromosomes 13 ( $P = 0.0013$ ) and 21 ( $P = 0.0015$ ), with no significant increase in the subgroup of patients  $\geq 37$  years of age. Anomalies in sex chromosomes were not significantly different from controls.

The pattern of repetition of chromosomal aberrations in subsequent IVF cycles was also analysed in 14 couples, who underwent at least two IVF attempts. A boxplot graphic (not shown) of intra-patient differences between the first and the second cycle shows a normal symmetric distribution and a median reaching zero ( $P = 0.905$ ; not significant), indicating a

similar proportion of abnormal embryos in each case in repetitive attempts.

We were able to follow embryo development up to the blastocyst stage (day 5) in 455 embryos from RM couples biopsied on day 3. As shown in Figure 1, there was a significantly ( $P < 0.0001$ ) higher percentage of euploid embryos reaching blastocyst stage as compared with the chromosomally abnormal embryos (61.7 versus 24.9%). Mosaic embryos, in which the two blastomeres analysed displayed discordant results, followed a pattern similar to normal embryos, with 56.8% reaching blastocyst stage on day 5 (most of them with one euploid blastomere combined with either an aneuploid or 1n/3n/4n blastomeres). On the other hand, embryos in which the biopsied blastomeres showed multinucleation were mostly arrested on days 3 and 4 of embryo development.

A detailed analysis of the effect of the different chromosomal abnormalities in embryo development (Table IV) revealed that among aneuploidies, autosomal monosomies were more detrimental, with only 20.2% forming blastocysts on day 5 ( $P < 0.0001$  versus normal embryos and versus

**Table IV.** Chromosomal abnormalities and embryo development in recurrent miscarriage patients

	<i>n</i> (%) Arrested day 3	Arrested day 4	Arrested day 5	Morula day 5	Blastocyst day 5	Total
Normal	0	11 (8.3)	2 (1.5)	38 (28.6)	82 (61.7)	133
Autosomal monosomy	5 (4.0)	48 (38.7) <sup>a</sup>	13 (10.5) <sup>b</sup>	33 (26.6)	25 (20.2) <sup>cp</sup>	124
Monosomy X	0	2 (18.2)	1 (9.1)	2 (18.2)	6 (54.5)	11
Trisomy	1 (1.4)	18 (25.0) <sup>d</sup>	14 (19.4) <sup>e</sup>	14 (19.4)	25 (34.7) <sup>fp</sup>	72
Monosomy/trisomy	1 (5.9)	8 (47.1) <sup>e</sup>	1 (5.9)	3 (17.6)	4 (23.5) <sup>h</sup>	17
Haploidy	1 (3.6)	13 (46.4) <sup>i</sup>	3 (10.7) <sup>j</sup>	8 (28.6)	3 (10.7) <sup>k</sup>	28
Triploidy	1 (20.0) <sup>l</sup>	2 (50.0) <sup>m</sup>	0	0	1 (25.0)	4
Tetraploidy	0	4 (80.0) <sup>n</sup>	0	0	1 (20.0) <sup>o</sup>	5

Comparisons versus normal embryos: <sup>a,c,e,g,i,k</sup> $P < 0.0001$ ; <sup>b</sup> $P = 0.0025$ ; <sup>d</sup> $P = 0.0015$ ; <sup>f</sup> $P = 0.0003$ ; <sup>h</sup> $P = 0.0037$ ; <sup>i</sup> $P = 0.0373$ ; <sup>l</sup> $P = 0.0292$ ; <sup>m</sup> $P = 0.0449$ ; <sup>n</sup> $P = 0.0004$ ; <sup>o</sup> $P = 0.00389$ .

Comparison monosomies versus trisomies: <sup>p</sup> $P < 0.0001$

trisomies), whereas embryos carrying monosomy X developed similarly to normal embryos. Trisomies also impaired embryo development, with 34.7% blastocyst formation ( $P = 0.0003$  versus normal embryos). Concerning the ploidy of the embryos analysed, most haploid embryos were arrested before cavitation, and only 10.7% reached blastocyst stage ( $P < 0.0001$  versus normal embryos). Triploid and tetraploid embryos also had lower rates of development, with 25.0 and 20.0% respectively reaching blastocyst stage.

## Discussion

The results of the present study confirm our preliminary findings (Simón *et al.*, 1998; Vidal *et al.*, 1998; Pellicer *et al.*, 1999): couples with RM produce chromosomally abnormal embryos in a significantly higher percentage than those not having this reproductive problem. Moreover, in 22% of these couples, the incidence of chromosomal aberrations affects all the embryos, and the percentage of abnormal embryos is similar in subsequent attempts. Therefore, after appropriate fertility work-up has ruled out other causes of RM, PGD is advisable not only as a therapeutic, but also as a diagnostic, tool.

Therapeutically, the results are not totally comparable with the control group, because in the latter most of the embryo replacements were performed on day 3. Thus, we may be comparing the implantation ability of a day 3 embryo versus a blastocyst, which is not the purpose of this study. We want to point out that an acceptable implantation rate per embryo replaced was reached in the study group (28%), providing evidence that PGD does not damage the embryos and that it can be safely and successfully employed to achieve a term pregnancy in these couples.

After PGD, we still observed three miscarriages; however, the results were acceptable in terms of miscarriage rate (13%) for a population of recurrent aborters. In one case, the embryo had a trisomy for chromosome 15, and the patient was 39 years old with an additional risk factor for aneuploidy (Gianaroli *et al.*, 1999; Kahraman *et al.*, 2000). We did not screen for this particular chromosome, although it has recently been reported that is frequently found in specimens from spontaneous abortions (Stephenson *et al.*, 2002). This fact emphasizes the need for new techniques that are able to screen for the entire set

of chromosomes, such as comparative genomic hybridization (Wilton *et al.*, 2001), but also raises the question of what would be the actual incidence of chromosomal abnormalities found in human embryos if the entire karyotype could be analysed.

The other two cases of spontaneous miscarriage after replacement of euploid embryos were three normal males. Among the genetic factors, highly skewed X chromosome inactivation has been found in patients with unexplained RM, suggesting that they could carry lethal X-linked mutations responsible for the lower rate of male offspring in these couples (Lanasa *et al.*, 2001), or X chromosomes with cryptic structural aberrations not identified even by high resolution banding (Uehara *et al.*, 2001). Therefore, either they were abnormal for other genetic factors not tested here, or they stopped growing for other reasons, emphasizing the need for a wider infertility work-up in RM couples.

It is also important to stress the high incidence of chromosomal abnormalities found in human embryos grown in the laboratory. The present data confirm that as many as 33% of embryos from young healthy women undergoing IVF will be chromosomally abnormal for the seven chromosomes tested. This rate doubles when age increases to  $\geq 37$  years, but in the presence of additional problems, such as RM or translocations (ESHRE PGD Consortium Steering Committee, 2002), the figure rises to 70%. The question is whether the environmental conditions of IVF (Natale *et al.*, 2001) or the process of ovarian stimulation (Viuff *et al.*, 2001) may cause a substantial number of these abnormalities. In order to answer this question, a comparison must be made with natural conceptions, since this would explain not only the numbers found using FISH for PGD, but also the low success rates of assisted reproduction technology, and perhaps also the increased risk of malformations recently found in children derived from these techniques (Hansen *et al.*, 2002).

The origin of autosomal trisomies has been investigated, and several studies using DNA polymorphism have revealed non-disjunction during maternal meiosis, usually associated with maternal age (Nicolaidis and Petersen, 1998; review). A similar meiotic behaviour could be responsible of the autosomal monosomies and trisomies found in the preimplantation embryos of RM couples. In fact, the success of oocyte donation in women with RM supports the idea that the oocyte

may be the origin of infertility in most of these couples (Remohí *et al.*, 1996). However, the origin of the single X in monosomy for the X chromosome is usually maternal (80%), implying a paternal error during meiosis (Chandley, 1981). In 50% of 47,XXY and in 100% of 47,XYY, the origin is paternal non-disjunction (Jacobs and Hassold, 1995). In this sense, FISH studies in the sperm of couples with RM have shown an increased incidence of sex chromosome disomy and diploidy in seven out of 40 sperm samples from couples with unexplained recurrent miscarriage (Rubio *et al.*, 1999). The abnormal behaviour of centromeres has also been suggested to predispose to meiotic non-disjunction, affecting all chromosomes in couples with RM (Bajnoczky and Gardó, 1993). This last report agrees with our data, in which chromosome-specific aneuploidy was not observed.

Another important issue is the frequency of chromosomal abnormalities in RM. A recent study has reported 29% of abnormal karyotypes in 167 patients with 3–16 miscarriages before 20 weeks (Carp *et al.*, 2001). These authors found that after an aneuploid miscarriage, there was a 68% live birth rate for a subsequent pregnancy compared with 41% after an euploid miscarriage. These results contrast with the high prevalence of aneuploidy observed in the preimplantation embryos of our study. To understand these differences, two important issues should be taken into account: the relationship of the number of previous abortions and gestational age with the risk of chromosomal abnormalities. The frequency of abnormal embryonic karyotypes found in spontaneous abortions has been inversely correlated with the number of previous miscarriages (Ogasawara *et al.*, 2000), with a higher incidence in couples with two to three miscarriages and decreasing with the number of previous abortions. Other authors have reported a lower incidence of euploid pregnancies and higher frequency of trisomies in embryonic losses (6–10 weeks) compared with preclinical (<6 weeks) and fetal losses (10–20 weeks) (Stephenson *et al.*, 2002). In our study, most of the couples were in the group with 2 or 3 previous embryonic losses and PGD would be indicated to improve their reproductive outcome.

Concerning in-vitro embryo development, Almeida and Bolton reported the effect of chromosomal abnormalities in the first steps from fertilization to the 5- to 8-cell stage (Almeida and Bolton, 1996). With the introduction of FISH to the IVF setting, there have been more reports regarding the relationship of embryo morphology and development to chromosomal abnormalities. Magli *et al.* found that only 21.9% of embryos diagnosed as abnormal on day 3 reached blastocyst stage, versus 34.3% of normal embryos (Magli *et al.*, 2000). Similar results have been reported in FISH studies in the blastocyst (Sandalinas *et al.*, 2001). Interestingly, a low percentage of monosomies was found at the blastocyst stage, and an important percentage of trisomic embryos progressed to form blastocysts, agreeing with the results observed in spontaneous abortions.

The present and the above-mentioned reports have clearly described the ability of human embryos carrying numerical chromosome abnormalities to develop to the blastocyst stage. The interesting finding in our report is that we have focused our

analysis on embryos derived from patients with RM. It is worth mentioning that, in contrast to Sandalinas *et al.* who found that only 9% of monosomies reached blastocyst stage (Sandalinas *et al.*, 2001), in our series autosomic and X monosomies developed to the blastocyst in 20 and 55% of cases respectively. The same is true for mosaicisms, in which we have described a potential to develop to blastocysts that is similar to that of normal embryos.

What is the meaning of this difference? Are we dealing with couples capable of producing abnormal embryos that for some reason continue development and implant, whereas in the normal fertile population they stop growing? Perhaps this is the explanation, since monosomy X is also one of the most frequent chromosomal anomalies found in products from spontaneous abortions. However, we should also bear in mind that the culture systems employed in each report were different, and we know that environmental factors can play an important role in embryo development (Natale *et al.*, 2001). Perhaps we are just observing that our co-culture system (Simón *et al.*, 1999) is able to more successfully grow normal and abnormal embryos to the blastocyst stage, and this is why our rates of blastocyst development seem higher than with commercially available sequential media. Higher blastocyst rates have also been reported in poor quality embryos co-cultured with human Fallopian ampullary cells compared with culture medium alone (Weichselbaum *et al.*, 2002).

Trisomies also developed to blastocysts in 34.7% of cases. Polyploidies arrested early, again providing an explanation for the early findings in women with spontaneous abortions (Boué *et al.*, 1975; Hassold *et al.*, 1978; Plachot, 1989; Eiben *et al.*, 1990; Stephenson *et al.*, 2002). Therefore, under normal conditions nature provides a quality control for human embryos in the very early stages of development. Under other conditions, however, the products of conception are rejected later in pregnancy, resulting in a clinical abortion.

In summary, the results of the present study are reassuring in the sense that couples with RM display more abnormal embryos *in vitro* than couples without this problem. Moreover, many of these embryos (especially monosomy X and mosaics) are able to develop *in vitro*, providing support for the introduction of PGD to the diagnostic and therapeutic arsenal for the treatment of couples with RM, and also giving a logical explanation to the type of chromosomal anomalies found in specimens from spontaneous abortions.

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