

## OUTSTANDING CONTRIBUTION

# Positive outcome after preimplantation diagnosis of aneuploidy in human embryos\*

Santiago Munné<sup>1,4</sup>, Cristina Magli<sup>2</sup>, Jacques Cohen<sup>1</sup>, Paula Morton<sup>3</sup>, Sasha Sadowy<sup>1</sup>, Luca Gianaroli<sup>2</sup>, Michael Tucker<sup>3</sup>, Carmen Márquez<sup>1</sup>, David Sable<sup>1</sup>, Anna Pia Ferraretti<sup>2</sup>, Joe B.Massey<sup>3</sup> and Richard Scott<sup>1</sup>

<sup>1</sup>Institute for Reproductive Medicine and Science, Saint Barnabas, Livingston, NJ, <sup>2</sup>S.I.S.Me.R, Bologna, Italy, <sup>3</sup>Reproductive Biology Associates, Atlanta, GA, USA

<sup>4</sup>To whom correspondence should be addressed at: The Institute for Reproductive Medicine and Science, Saint Barnabas Medical Center, 101 Old Short Hills Rd, Suite 501, Livingston, NJ 07052, USA

**Chromosomal abnormalities are responsible for a great deal of embryo wastage, which is reflected, at least partially, in decreased implantation and increased miscarriage in older women. To address this problem the transfer of only chromosomally normal embryos previously selected by preimplantation genetic diagnosis (PGD) has been proposed. We designed a multi-centre in-vitro fertilization (IVF) study to compare controls and a test group that underwent embryo biopsy and PGD for aneuploidy. Patients were matched retrospectively, but blindly, for average maternal age, number of previous IVF cycles, duration of stimulation, oestradiol concentrations on day +1, and average mature follicles. All these parameters were similar in test and control groups. Only embryos classified as normal for those chromosomes were transferred after PGD. The results showed that the rates of fetal heart beat (FHB)/embryo transferred between the control and test groups were similar. However, spontaneous abortions, measured as FHB aborted/FHB detected, decreased after PGD ( $P < 0.05$ ), and ongoing pregnancies and delivered babies increased ( $P < 0.05$ ) in the PGD group of patients. Two conclusions were obtained: (i) PGD of aneuploidy reduced embryo loss after implantation; (ii) implantation rates were not significantly improved, but the proportion of ongoing and delivered babies was increased.**  
*Key words:* in-vitro fertilization/preimplantation genetic diagnosis/trisomy 21, 18, 13, 16

## Introduction

The causes of the decline in implantation observed with increasing maternal age are still debated. The high implantation rate in women >40 years old observed after transfer of embryos from younger women strongly suggests that their ability to become pregnant is largely unaffected, whereas their oocyte quality is compromised (Navot *et al.*, 1994). Altered oocyte metabolism such as ATP production (Van Blerkom, 1995) and excessive deposition of zona pellucida glycoproteins (Garside *et al.*, 1997) have been associated with advanced maternal age. More important, genetic analysis of aborti and live offspring have shown that older women are at a higher risk of conceiving trisomic fetuses and that most of these are maternal in origin (Hassold and Chiu, 1985; Warburton *et al.*, 1986; Antonorakis *et al.*, 1991; Fisher *et al.*, 1995). Similarly, a significant increase in aneuploidy with maternal age was found in both unfertilized oocytes (Dailey *et al.*, 1996) and cleavage-stage embryos (Munné *et al.*, 1995a,b). The rate of chromosomal abnormalities in embryos is considerably higher than the one reported in spontaneous abortions, suggesting that a considerable proportion of chromosomally abnormal embryos are eliminated before any prenatal diagnosis. Such loss may partly account for the decline in implantation in older women. For instance, the rate of embryonic monosomy is similar to the one for trisomy, whereas, with the exception of monosomy 21 (1/1000 karyotyped abortions), autosomal monosomies are normally undetected in pregnancy (Munné *et al.*, 1995a). Other evidence comes from the observations that blastocyst formation declines with maternal age in women >30 years, and that more embryos arrest at the morula stage, being possibly monosomic or developmentally affected (Janny and Ménézo 1996).

Because of the association between aneuploidy and implantation, it was postulated that selection of chromosomally normal embryos could reverse this trend (Munné *et al.*, 1993). However, while some research groups felt that this was possible and desirable (Verlinsky and Kuliev, 1996; Gianaroli *et al.*, 1997) others doubted its value or feasibility (Egozcue, 1996; Reubinoff and Sushan, 1996). Currently, removal of aneuploid embryos can only be done through PGD, after either polar body or blastomere analysis. Fluorescence in-situ hybridization (FISH) allows chromosome enumeration on interphase cell nuclei, i.e. without the need for culturing cells or preparing metaphase spreads. FISH has been applied to preimplantation genetic diagnosis (PGD) of common aneuploidies (at least XY, 13, 18, 21), testing either human blastomeres from cleavage-

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stage embryos or oocyte polar bodies (Munné *et al.*, 1993, 1995a,b, 1998a,b; Manor *et al.*, 1996; Verlinsky *et al.*, 1995, 1996b; Gianaroli *et al.*, 1997). More than 500 cycles have been performed using this technique, resulting in >100 chromosomally normal babies. However, an increase in implantation rate or a decrease in abnormal offspring has not been demonstrated, either for lack of data or appropriate controls. Here we present the first study including carefully matched retrospective controls to analyse whether PGD of aneuploidy has any effect on implantation rates, spontaneous abortions, and live births.

## Materials and methods

### Clinical cases

PGD was performed in three centres. Written consent was obtained from patients in accordance with each internal review board protocol. Inclusion of patients followed these criteria: maternal age was  $\geq 35$  years; at least one embryo was replaced; pregnancy information was available at least up to the 20th week of pregnancy; and all replaced embryos were classified as chromosomally normal.

### Patient matching

Patients 35 years or older undergoing in-vitro fertilization (IVF) treatment at the three centres participating in this study were offered PGD of aneuploidy. Randomization was initially offered but, because there was no data supporting a beneficial effect of PGD of aneuploidy, few patients agreed to the study and those committed to it rejected randomization. Therefore, the study was planned as a retrospective study where controls were matched to test patients blindly or previous pregnancy outcome was known. PGD cases for centres 2 and 3 were matched, as far as was possible, on the following variables which are listed in order of matching priority: maternal age ( $\pm 1$  year); concentration of oestradiol (with a difference between control and test of  $\pm 20\%$ ); month of retrieval; duration of stimulation; and number of mature follicles. The rationale for matching them according to these factors, in addition to maternal age, was to avoid changes in laboratory performance and to control for ovarian reserve and response (Scott and Hofmann, 1995). Patients from centre 1 were also matched according to the number of previous IVF attempts. This extra condition is included because in that centre implantation significantly decreases between the first and second attempt. This phenomenon was not observed in many other centres (see review Meldrum *et al.*, 1998b).

The controls also fulfilled the requirements that at least one embryo was replaced and that pregnancy information was available at least up to the 20th week of pregnancy.

### Biopsy, fixation and fish procedure

During day 3 of development, one or two cells per embryo were biopsied, and the embryos returned to culture as described elsewhere (Grifo, 1992). All of the embryos were at the 4–12-cell stage of development at the time of biopsy. Most embryos classified as normal after PGD were transferred to the uterus on the same day of analysis. All blastomeres were fixed individually following our protocol (Munné *et al.*, 1996).

The present study has taken 2 years, during which fluorescence in-situ hybridization (FISH) protocols for PGD of aneuploidy have evolved considerably. PGD cases included in this study were therefore performed using the protocols available at the time. Some cases used probes for simultaneous detection of chromosomes X, Y, 18 and the shared alpha-satellite region of chromosomes 13 and 21 (Munné *et al.*, 1993) ( $n = 14$ ). Later on, cases used specific probes for X, Y, 13, 18

and 21 (Munné *et al.*, 1996) ( $n = 22$ ). Later still, a probe for chromosome 16 was added to the previous mixture (Munné *et al.*, 1998a) and used in a proportion of the cases ( $n = 50$ ). And finally, a small fraction of cases ( $n = 31$ ) benefited from having the biopsied cells analysed with the X, Y, 13, 16, 18, and 21 probe mixture (Munné *et al.*, 1998b), and re-analysed with a second probe mixture specific for chromosomes 14, 15 and 22. Thereby all the cases had their biopsied cells analysed for the trisomies with potential of arriving to term (X, Y, 13, 18, 21), and some for trisomies commonly found in spontaneous abortions, but which do not survive to term (14, 15, 16, 22). The protocols for these probe sets have been previously published (Munné *et al.*, 1993, 1996, 1998a,b; Munné and Weier, 1996) and a few of the cases here reported were also included in previous manuscripts (Munné *et al.*, 1996, 1998a,b).

The FISH error rate has already been evaluated in previous studies of probes for X, Y, 18, 13/21 (7%) (Munné *et al.*, 1993); X, Y, 13, 18, 21 (13%) (Munné and Weier, 1996); X, Y, 13, 16, 18, 21 (9%) (Munné *et al.*, 1998a); and X, Y, 13, 16, 18, 21, 14, 15, 22 (15%) (Munné *et al.*, 1998b). The scoring criteria (described in Munné and Weier, 1996) were applied in any cases that used at least probes specific for X, Y, 13, 18 and 21 chromosomes.

### Statistical analysis

The patients in the two treatment groups were matched, primarily by age, but also to a lesser extent by oestradiol and cycles of IVF. This was to avoid any potential bias caused by these variables.

The data were analysed in the following way. For each patient the statistic of interest (say FHB/transfer) was calculated, and from that the mean values and standard errors were calculated. Thus the 'error' reflects inter-patient variation. This is a more appropriate way to analyse the data than by counting embryos as units. Therefore, following the example of the variable 'FHB/transfer', the mean for that variable would not be the sum of all FHB divided by the total number of embryos transferred. The variables of interest were generally proportions based on very small frequencies, so that great care was required in the statistical analysis.

Since the variables of interest were proportions, the main method of analysis was logistic regression. The variables contributing to the proportions of interest (for example number of FHB/transfer) were entered into the GLM (generalized linear modelling) analysis, which then tested for systematic differences caused by treatment and centre. Maternal age was also included as a covariate, but, because of the matching process described above, this had minimal impact. The analysis generated estimated mean values and standard errors for the various sub-groups, these figures being quoted in the tables. The statistical tests were carried out within the algorithm, and the findings are also quoted in the tables. The algorithm used to carry out the calculations was Genstat (1988).

## Results

### Fitness of controls

Women ( $n = 117$ ) undergoing PGD for aneuploidy were matched with controls ( $n = 117$ ). The parameters for which the cases were matched and the outcome for each of the 234 patients are available from the authors (see Appendix). The matching procedure was successful in that the relevant mean values for the two treatment groups were fairly similar, as may be seen below. Maternal age in both test and control groups averaged 38.5 (mean  $\pm 0.23$  SD). The duration of stimulation averaged 11.7 (mean  $\pm 3.2$  SD) days in test and

**Table I.** Statistical analysis of the results

Variable	Test group	Control group	<i>P</i>
Sacs/embryos transferred <sup>a</sup>	0.187 ± 0.021	0.156 ± 0.018	NS
FHB/embryos transferred <sup>a</sup>	0.176 ± 0.020	0.137 ± 0.017	NS
Fetus aborted/FHB <sup>a</sup>	0.096 ± 0.037	0.242 ± 0.056	0.05
(Fetus aborted + blighted ova)/sacs <sup>a</sup>	0.150 ± 0.043	0.338 ± 0.057	0.05
Ongoing + delivered/embryos transferred <sup>a</sup>	0.159 ± 0.020	0.106 ± 0.015	0.05
Mean no. initial pregnancies/patient	0.359 (42/117)	0.299 (35/117)	NS
Mean no. abortions/patient	0.143 (6/42)	0.257 (9/35)	NS
Mean no. ongoing pregnancies/patient	0.325 (38/117)	0.222 (26/117)	NS

<sup>a</sup>Values are mean numbers ± SD.

NS = not significant; FHB = fetal heart beat.

**Table II.** Results by centre

	Centre	Case	Control
Sacs/embryos transferred	1	0.206 ± 0.032	0.180 ± 0.030
	2	0.207 ± 0.039	0.184 ± 0.034
	3	0.132 ± 0.036	0.085 ± 0.026
FHB/embryos transferred	1	0.193 ± 0.032	0.174 ± 0.030
	2	0.198 ± 0.038	0.155 ± 0.031
	3	0.121 ± 0.034	0.060 ± 0.022
Fetus aborted/FHB	1	0.137 ± 0.064	0.274 ± 0.087
	2	0.087 ± 0.060	0.100 ± 0.067
	3	0	0.445 ± 0.192
(Fetus aborted + blight ova)/sacs	1	0.192 ± 0.071	0.305 ± 0.086
	2	0.128 ± 0.069	0.244 ± 0.087
	3	0.085 ± 0.081	0.607 ± 0.155
Ongoing + delivered/embryos transferred	1	0.167 ± 0.030	0.124 ± 0.026
	2	0.179 ± 0.037	0.139 ± 0.030
	3	0.120 ± 0.034	0.034 ± 0.016

Values are means ± SD.

FHB = fetal heart beat.

11.1 (mean ± 3.2 SD) days in controls. The average mature follicles numbered 12.6 (mean ± 6.7 SD) (test) and 12.4 (mean ± 5.8 SD) (controls). In addition, for centre 1, the average number of repeated IVF cycles was 1.9 (mean ± 1.2 SD) cycles (test) and 1.8 (mean ± 1.0 SD) cycles (controls). Oestradiol concentrations on day ±1 were measured with different systems in each centre, therefore an average is not given. Nevertheless, there was no statistical evidence of a systematic difference between test and controls regarding oestradiol or any other parameter.

### Pregnancy outcome

The overall results and statistical analysis are shown in Table I. Implantation rates, as FHB/embryo, were slightly higher in the PGD group (17.8%) but not statistically different from the control group (13.7%). However, spontaneous abortions (lost FHB/FHB detected) decreased 2.5-fold after PGD ( $P < 0.05$ ), from 23% in control to 9% in the test group. The slight increase in implantation and the significant decrease in spontaneous abortions resulted in a significant increase ( $P < 0.05$ ) in ongoing and delivered babies, from 10.5% (43/408) in controls to 16.1% (57/354) in the PGD group. When the experimental data were summarized as the number of patients becoming pregnant, or miscarrying, the trends were similar to those observed in Table I, although the findings were not statistically significant. It would appear therefore that the additional information contained in

variables such as 'number of transfers' was necessary in order to highlight the treatment differences. Also the results derived from the rather complex GLM (general linear modelling) method of Table I could often be replicated by a more simple approach. Thus if the ratio (ongoing/transfer) was calculated for each patient, the mean ± SD for the PGD group was  $0.157 \pm 0.024$ , and for the controls was  $0.107 \pm 0.021$ , values very similar to those quoted in Table I. Furthermore, working with pairs of matched patients, we found that in 66 the same ratio was obtained, in 32 pairs the PGD ratio was greater than the control ratio, and in 19 pairs the control ratio was greater than the experimental group ratio. Therefore, of the 51 pairs where there was a non-zero difference in the ratios, the PGD ratio was the higher in 32 of those pairs. The departure from the expected value under the null hypothesis (25.5) was just significant at the 5% level, thus supporting the finding in Table I. Thus by adopting a completely different, non-parametric approach of counting differences, we obtained precisely the same result as the GLM analysis used to produce Table I.

Table II shows the results obtained when calculated per centre. Because the numbers of cases were often quite small, it was not possible to employ a matched procedure, and these results are derived from an unpaired investigation. Although the trends were similar at the three centres and consistent with the overall results, the number of cases was not sufficient to provide a conclusive statistical finding.

When the data were analysed by probe combination used we found a higher decrease in spontaneous abortions when using XY, 13, 14, 15, 16, 18, 21, 22 probe combination (from 29% in controls to 4% in test group) than when other probes were used (20% in controls to 12.5% in test group). Implantation rates appeared to increase only when the XY, 13, 14, 15, 16, 18, 21, 22 probe combination was used, increasing from 18% in controls to 31% in the test group. However, due to the small sample size, these differences were not statistically different.

Analysis during prenatal diagnosis and after birth showed no abnormalities in the test group for the chromosomes assessed. However, a spontaneous abortion was shown to be trisomy 21. The cells belonging to the transferred embryos of the case resulting in trisomy 21 were re-hybridized with a probe for chromosome 21 (already described in a previous study) (Munné *et al.*, 1998b) and the same normal result was obtained.

## Discussion

Preimplantation genetic diagnosis of aneuploidy using probes for four to eight chromosome pairs was performed in 117 couples of advanced maternal age. Their pregnancy results were then compared blindly and retrospectively to 117 controls matched by age, days of hormonal stimulation, oestradiol concentrations, and, for centre 1, repeated IVF cycles. The results indicated an increase in implantation rates, although not statistically significant, from 14% in the test group to 18% in the PGD group. Spontaneous abortions decreased 2.5-fold, from 23% in controls to 9% in the PGD group, which, combined with the slight increase in implantation rate in the PGD group, produced a significant increase in ongoing and delivered babies from 11% in controls to 16% in the PGD group.

From the present results, it can be postulated that aneuploidy determination prior to embryo transfer may reduce the incidence of miscarriage, and increase the rate of delivery. Because the chromosomes analysed in this study are involved in at least three-quarters of chromosomally abnormal miscarriages (Schmidt-Sarosi *et al.*, 1998), a reduction in pregnancy loss should be expected, and emphasizes the accuracy of the proposed hypothesis. It is important to consider the conditions under which these results were obtained, emphasizing the need for further, more advanced studies. The results were evaluated retrospectively and obtained in three different centres, during a 3 year period. During this period, conditions of assisted reproduction changed, most notably in the use of follicular stimulation protocols, but also because other probes became available. An increase in ongoing pregnancies and live births was most notably due to a reduction of the occurrence of miscarriage.

In most IVF centres there is a reduction of ~30–50% in implantation rate from patients 25–34 to 35–42 years old. If such decrease were solely produced by chromosome abnormalities, we would expect an increase in implantation after PGD of a similar magnitude, provided sufficient number of oocytes were available, which is not the case. The low PGD effect on implantation can be attributed to at least three factors. One could be that the chromosomes chosen for this test are those producing abnormalities compatible with further development. For instance, trisomy 1 is found in cleavage stage embryos but never

detected in spontaneous abortions (Watt *et al.*, 1987; Simpson 1990; Delhanty *et al.*, 1997; Laverge *et al.*, 1997), and other chromosomes, such as 17, seldom found in spontaneous abortions, are quite common in cleavage-stage embryos (Simpson, 1990; Bahçe *et al.*, 1999). The importance of the probes used is also evident when comparing PGD cases performed with different probe mixtures, with the one used to test eight chromosomes producing the highest implantation rate improvement and the highest reduction in spontaneous abortions.

A second factor would be that the working hypothesis is incorrect, namely, in opposition to previously held ideas, chromosomally abnormal embryos may in fact often implant, many of them dying shortly afterwards and being detected as empty sacs. This would also imply that embryos of women of advanced maternal age may be less likely to implant because of non-chromosomal causes.

The third factor affecting implantation might be the embryo biopsy procedure. Although a previous study on embryo biopsy did not show a negative effect on embryo development to blastocyst (Hardy *et al.*, 1990), embryo transfer effects, if any, were not assessed. Moreover, the embryos studied were from donors and not from older patients or those with a poor prognosis. Evidence of embryo damage during transfer of zona-drilled embryos comes from assisted hatching studies. For instance, assisted hatching has been demonstrated to increase implantation rates (Cohen *et al.*, 1990), but its potential is related to the diameter of the opening in the zona. Openings of 40–60 µm diameter commonly used for biopsy, instead of the recommended 20–30 µm for assisted hatching, might eliminate any beneficial effect (B.Schoolcraft and T.Schlenker, personal communication), and cause embryo damage immediately after the procedure or during and after embryo transfer. Preliminary data from one of the centres reporting in this study, compared two embryologists performing embryo biopsies, one with zona openings 40–50 µm in diameter and the other 30–40 µm. The first obtained a 13% (22/166) implantation rate per embryo transferred compared to 21% (16/76) of the second embryologist. Although the differences were not significant, they point again to a very serious detrimental effect of large zona openings. Another effect of embryo biopsy could be the toxicity of acidified Tyrode's solution on the adjacent blastomeres, although the appearance of effective laser devices could make its use obsolete (Montag *et al.*, 1998). Other negative effects of embryo biopsy may be that it interferes with cell allocation and positioning during compaction and blastulation (Edwards and Beard, 1998). However, embryos with 15% fragments and seven cells on day 3, have the same implantation rate as 8-cell embryos when the fragments have been removed (Alikani *et al.*, 1999), and these embryos could be compared to biopsied embryos in which one cell has been removed. Similarly, linear tracing experiments have demonstrated that any cell of an 8-cell embryo could produce ICM (Mottla *et al.*, 1995). A more clear negative effect of embryo biopsy could be the interference with compaction. For instance, data from centre 1 indicates that there is a 4% decrease in embryo implantation when the biopsy is performed 70 h or later after embryo retrieval compared to when it is performed 67 h or earlier. Clearly, more research is needed on the effect of

embryo biopsy on implantation, when beneficial effects such as PGD are excluded.

The potential hazards of embryo biopsy could be avoided by using polar body instead of blastomere biopsy (Verlinsky *et al.*, 1995, 1996; Munné *et al.*, 1996). Polar body biopsy can be performed by mechanical or laser techniques and does not interfere with cell allocation. The hole in the zona is on average 10–20 µm, about the size recommended for AHA. If the polar body approach is taken, the issue of which probes to use might be solved using spectral imaging techniques (Schröck *et al.*, 1996) to karyotype all 23 chromosomes of the polar body as already demonstrated (Márquez *et al.*, 1998). Although most aneuploidies are maternal in origin, polar body analysis would not detect other abnormalities such as polyploidy, haploidy and mosaicism involving most or all cells and accounting for at least 19% of embryonic chromosome abnormalities (Munné *et al.*, 1995).

In conclusion, the present study demonstrates that PGD of aneuploidy can positively affect the outcome of embryos by reducing embryo wastage and increasing implantation rates. As PGD of aneuploidy now stands, patients of advanced maternal age undergoing IVF can benefit from this technique by avoiding the trauma of losing greatly desired pregnancies and giving themselves a greater chance of having a baby. Because the procedure appears beneficial, further research can continue in order to elucidate the causes behind the disappointing effects on implantation. We are currently investigating the use of other probes, spectral imaging, laser embryo biopsy and polar body biopsy in a randomized study to improve the procedure, hopefully to bring implantation rates in those women in line with the rates found in younger patients.

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#### Appendix. Matching characteristics and pregnancy outcome of test cases and controls

Match #	Centre	Maternal age (years)	Days stim.	Oestradiol (pg/ml)	Mature follicles (n)	Eggs retrieved (n)	IVF cycles (n)	Probe <sup>a</sup> solution	No. embryos transferred	No. sacs	No. blighted ova	Fetal heart beats	Spont. abortions (n)	Ongoing deliveries or reduced (n)
Test group														
1	1	40	10	2136	12	18	6	1	4	0	0	0	0	0
2	1	35	11	1674	7	23	1	1	4	3	0	3	1	2
3	1	40	10	3914	9	32	1	1	5	0	0	0	0	0
4	1	41	9	1973	13	16	1	1	5	4	0	4	0	4
5	1	36	9	2997	29	31	1	1	4	1	0	1	0	1
6	1	36	9	2731	15	21	4	1	4	0	0	0	0	0
7	1	40	8	2209	7	12	1	1	3	1	0	1	0	1
8	1	40	8	4712	19	25	2	1	4	0	0	0	0	0
9	1	42	8	5596	14	17	2	1	4	0	0	0	0	0
10	1	40	11	1835	8	11	2	1	5	0	0	0	0	0
11	1	39	10	3062	19	18	1	1	5	1	0	1	0	1
12	1	39	11	4852	20	27	2	1	5	1	0	1	0	1
13	3	40	9	3108	27			1	5	0	0	0	0	0
14	3	39	12	1657	7			1	4	1	0	1	0	1
15	1	42	9	3693	11	22	2	2	6	0	0	0	0	0
16	1	39	10	2864	15	33	2	2	5	1	0	1	1	0
17	1	35	12	1550	5	3	1	2	3	2	0	2	0	2
18	1	43	9	2629	15	24	1	2	4	0	0	0	0	0
19	1	35	11	1992	10	19	4	2	4	0	0	0	0	0
20	1	42	7	3358	15	12	2	2	6	0	0	0	0	0
21	1	41	10	2967	21	31	1	2	2	0	0	0	0	0
22	1	35	10	4395	17	28	4	2	4	0	0	0	0	0
23	1	40	11	1684	12	19	1	2	3	1	0	1	0	1
24	1	35	13	3269	14	12	2	2	4	0	0	0	0	0
25	1	36	11	1568	6	5	1	2	3	1	0	1	0	1
26	3	36	12	1127	7			2	1	0	0	0	0	0
27	3	40	11	2128	4			2	1	0	0	0	0	0
28	3	42	12	2616	13			2	2	0	0	0	0	0
29	1	38	9	2994	26	25	1	2	5	1	0	1	0	1
30	2	39	18	1970	5			2	3	2	0	2	0	2
31	2	37	16	2331	7			2	3	2	1	1	0	1
32	2	39	18	1820	7			2	1	0	0	0	0	0
33	2	41	19	1295	5			2	5	1	0	1	0	1
34	2	35	15	2183	6			2	3	0	0	0	0	0
35	3	36	11	3286	18			2	3	1	0	1	0	1
36	3	41	12	2312	12			2	4	0	0	0	0	0
37	2	36	16	3687	13			3	3	0	0	0	0	0
38	2	36	15	2053	8			3	2	0	0	0	0	0
39	2	37	14	2677	7			3	4	0	0	0	0	0
40	2	38	15	790	5			3	3	2	0	2	0	2
41	2	38	17	861	5			3	3	0	0	0	0	0
42	2	36	17	2847	7			3	1	0	0	0	0	0
43	2	38	13	4752	9			3	2	0	0	0	0	0
44	3	39	11	2892	10			3	2	0	0	0	0	0
45	1	41	9	831	11	11	2	3	3	0	0	0	0	0
46	3	39	11	3162	14			3	3	2	0	2	0	2
47	3	42	12	3556	26			3	3	0	0	0	0	0
48	3	37	10	2703	11			3	2	0	0	0	0	0
49	3	39	12	2095	7			3	2	1	0	1	0	1
50	3	39	11	3434	14			3	4	0	0	0	0	0
51	2	38	16	3157	8			3	4	0	0	0	0	0
52	2	35	14	4248	8			3	2	1	0	1	1	0
53	2	35	15	2127	11			3	3	0	0	0	0	0

## Appendix. Continued

Match #	Centre	Maternal age (years)	Days stim.	Oestradiol (pg/ml)	Mature follicles (n)	Eggs retrieved (n)	IVF cycles (n)	Probe <sup>a</sup> solution	No. embryos transferred	No. sacs	No. blighted ova	Fetal heart beats	Spont. abortions (n)	Ongoing deliveries or reduced (n)
54	2	39	15	1803	7			3	3	2	0	2	0	2
55	2	39	16	1099	6			3	3	0	0	0	0	0
56	2	35	17	3422	11			3	4	0	0	0	0	0
57	2	40	16	1513	8			3	3	0	0	0	0	0
58	1	37	10	2796	13	17	1	3	3	1	0	1	0	1
59	1	38	11	1727	10	10	1	3	2	0	0	0	0	0
60	3	39	12	2148	10			3	1	0	0	0	0	0
61	1	40	9	1756	10	9	2	3	3	0	0	0	0	0
62	1	42	8	4455	23	20	2	3	4	1	0	1	1	0
63	3	43	10	4884	23			3	4	0	0	0	0	0
64	3	37	9	3332	13			3	4	3	0	3	0	3
65	2	39	10	259	2			3	2	0	0	0	0	0
66	2	38	11	748	6			3	2	0	0	0	0	0
67	2	38	11	2865	17			3	4	2	0	2	1	1
68	2	44	13	2111	9			3	1	0	0	0	0	0
69	2	37	13	1523	10			3	3	0	0	0	0	0
70	2	41	13	2119	8			3	1	0	0	0	0	0
71	2	35	12	294	3			3	1	0	0	0	0	0
72	1	37	9	2682	17	24	2	4	4	3	1	2	0	2
73	1	35	10	1492	13	14	3	3	3	0	0	0	0	0
74	3	37	12	2193	11			3	3	0	0	0	0	0
75	3	36	15	1696	40			3	3	0	0	0	0	0
76	3	35	12	1849	11			3	3	0	0	0	0	0
77	3	36	11	3926	23			3	3	1	1	0	0	0
78	3	37	12	2389	14			3	3	1	0	1	0	1
79	3	38	9	2948	25			3	4	1	0	1	0	1
80	3	35	10	2552	10			3	5	0	0	0	0	0
81	3	39	12	1621	11			3	2	0	0	0	0	0
82	3	38	12	3714	14			3	2	0	0	0	0	0
83	1	40	11	1524	8	15	1	3	3	0	0	0	0	0
84	3	45	9	3298	24			3	2	0	0	0	0	0
85	3	42	11	2796	11			3	4	0	0	0	0	0
86	1	38	11	3479	19	21	1	4	3	0	0	0	0	0
87	2	43	11	2076	15			4	3	0	0	0	0	0
88	2	36	12	1901	12			4	3	0	0	0	0	0
89	2	37	12	3152	15			4	2	1	0	1	0	1
90	2	40	12	3325	15			4	3	2	0	2	0	2
91	2	41	13	826	13			4	3	0	0	0	0	0
92	2	43	14	510	10			4	1	0	0	0	0	0
93	2	39	15	2846	14			4	3	3	0	3	0	3
94	3	40	9	2480	15			3	4	0	0	0	0	0
95	3	38	10	1781	8			4	2	0	0	0	0	0
96	1	40	9	4913	17	37	4	4	3	0	0	0	0	0
97	3	38	10	4430	10			4	3	1	0	1	0	1
98	1	39	na	na	na			4	1	0	0	0	0	0
99	3	38	11	3640	11			4	3	0	0	0	0	0
100	1	39	8	3886	27	29	1	4	2	1	0	1	1	0
101	2	41	13	1691	16			4	3	2	0	2	0	2
102	2	35	13	1417	4			4	1	0	0	0	0	0
103	2	41	13	2268	12			4	3	1	0	1	0	1
104	2	36	13	2900	20			4	3	1	0	1	0	1
105	2	41	14	1390	12			4	2	1	0	1	0	1
106	2	41	14	1820	11			4	1	0	0	0	0	0
107	2	35	14	3150	18			4	3	0	0	0	0	0
108	2	43	15	1668	20			4	2	0	0	0	0	0
109	2	39	13	4031	12			4	1	0	0	0	0	0
110	2	38	15	384	3			4	2	0	0	0	0	0
111	1	40	7	762	8	10	2	4	2	1	0	1	0	1
112	1	36	8	2104	23	23	2	4	4	0	0	0	0	0
113	1	38	8	1315	7	7	1	4	1	1	0	1	0	1
114	1	35	8	979	6	6	1	4	2	2	1	1	0	1
115	1	39	10	2458	8	7	2	3	5	0	0	0	0	0
116	1	36	10	1356	7	7	1	4	2	1	0	1	0	1
117	1	35	8	2366	20	30	3	4	4	4	0	4	0	4

## Appendix. Continued

Match #	Centre	Maternal age (years)	Days stim.	Oestradiol (pg/ml)	Mature follicles (n)	Eggs retrieved (n)	IVF cycles (n)	Probe <sup>a</sup> solution	No. embryos transferred	No. sacs	No. blighted ova	Fetal heart beats	Spont. abortions (n)	Ongoing deliveries or reduced (n)
Control group														
1	1	40	8	2167	14	16	4		4	0	0	0	0	0
2	1	35	6	2101	12	24	1		5	0	0	0	0	0
3	1	41	10	3424	24	30	1		5	0	0	0	0	0
4	1	41	10	2018	8	20	1		5	2	0	2	0	2
5	1	36	10	3935	19	39	1		3	1	1	0	0	0
6	1	37	9	2321	16	18	4		4	0	0	0	0	0
7	1	40	9	1931	17	12	1		4	0	0	0	0	0
8	1	41	9	4024	18	30	2		4	2	0	2	2	0
9	1	42	8	3024	19	22	2		4	0	0	0	0	0
10	1	42	8	1324	12	10	2		5	1	0	1	1	0
11	1	40	7	1463	16	21	1		5	2	0	2	0	2
12	1	40	8	3320	17	32	2		5	0	0	0	0	0
13	3	40	10	2970	12				4	1	0	1	0	1
14	3	39	10	1984	20				4	0	0	0	0	0
15	1	42	8	3190	12	22	2		5	0	0	0	0	0
16	1	39	8	2524	16	35	2		5	0	0	0	0	0
17	1	35	7	1116	5	4	1		2	0	0	0	0	0
18	1	44	9	3415	15	21	1		2	0	0	0	0	0
19	1	36	7	2328	14	21	4		5	0	0	0	0	0
20	1	44	9	3415	15	21	1		2	0	0	0	0	0
21	1	41	8	7303	22	30	1		5	1	0	1	0	1
22	1	36	9	1938	18	26	4		4	1	0	1	1	0
23	1	40	10	2279	14	18	1		4	0	0	0	0	0
24	1	36	10	2491	9	11	2		5	0	0	0	0	0
25	1	36	11	747	6	5	1		3	0	0	0	0	0
26	3	37	11	1273	11				2	0	0	0	0	0
27	3	41	12	2433	12				4	0	0	0	0	0
28	3	41	12	2001	6				3	0	0	0	0	0
29	1	38	8	2295	16	25	1		4	2	0	2	0	2
30	2	39	13	1428	7				3	2	0	2	0	2
31	2	36	15	1819	11				4	0	0	0	0	0
32	2	39	13	817	5				4	1	0	1	0	1
33	2	42	15	1994	8				2	0	0	0	0	0
34	2	35	15	2605	15				4	3	0	3	0	3
35	3	35	11	3856	18				3	0	0	0	0	0
36	3	41	11	2908	14				5	1	1	0	0	0
37	2	36	20	1309	9				3	0	0	0	0	0
38	2	36	19	3551	5				3	0	0	0	0	0
39	2	37	16	1599	8				4	1	0	1	0	1
40	2	36	14	1535	10				3	2	0	2	0	2
41	2	40	14	1353	3				4	0	0	0	0	0
42	2	38	16	1749	9				4	0	0	0	0	0
43	2	39	11	1659	11				3	1	1	0	0	0
44	3	38	10	1929	6				4	0	0	0	0	0
45	1	41	10	3031	9	11	2		4	1	0	1	1	0
46	3	41	11	2524	16				4	0	0	0	0	0
47	3	39	11	2856	17				3	1	0	1	1	0
48	3	36	11	2112	7				3	0	0	0	0	0
49	3	38	12	2161	7				4	0	0	0	0	0
50	3	38	11	2716	19				4	0	0	0	0	0
51	2	38	14	1276	6				3	0	0	0	0	0
52	2	35	14	4132	12				4	0	0	0	0	0
53	2	35	14	2613	19				3	2	0	2	0	2
54	2	36	12	1439	11				2	2	0	2	0	2
55	2	38	17	1461	9				3	0	0	0	0	0
56	2	35	16	3263	10				4	0	0	0	0	0
57	2	40	14	800	7				3	3	1	2	0	2
58	1	38	8	3938	11	18	1		4	0	0	0	0	0
59	1	39	8	2547	21	11	1		4	1	0	1	0	1
60	3	38	11	1699	8				2	0	0	0	0	0
61	1	40	7	3387	12	10	2		3	0	0	0	0	0
62	1	42	10	1827	19	19	2		4	0	0	0	0	0
63	3	40	11	3730	11				4	0	0	0	0	0
64	3	37	10	3218	22				4	3	1	2	0	2
65	2	41	12	1060	4				4	0	0	0	0	0
66	2	37	9	894	4				3	0	0	0	0	0

## Appendix. Continued

Match #	Centre	Maternal age (years)	Days stim.	Oestradiol (pg/ml)	Mature follicles (n)	Eggs retrieved (n)	IVF cycles (n)	Probe <sup>a</sup> solution	No. embryos transferred	No. sacs	No. blighted ova	Fetal heart beats	Spont. abortions (n)	Ongoing deliveries or reduced (n)
67	2	36	13	2510	12				3	0	0	0	0	0
68	2	41	11	2146	4				3	0	0	0	0	0
69	2	38	12	1897	9				3	0	0	0	0	0
70	2	35	11	1460	15				3	0	0	0	0	0
71	2	38	11	372	4				3	1	0	1	0	1
72	1	38	8	2293	19	27	2		4	0	0	0	0	0
73	1	35	9	897	7	14	3		2	0	0	0	0	0
74	3	37	9	2937	27				4	0	0	0	0	0
75	3	36	12	2100	29				3	0	0	0	0	0
76	3	36	11	2788	20				3	0	0	0	0	0
77	3	35	12	4382	24				4	2	0	2	2	0
78	3	35	11	2512	19				4	0	0	0	0	0
79	3	39	13	2224	11				4	0	0	0	0	0
80	3	35	12	1732	11				4	0	0	0	0	0
81	3	39	10	1748	8				4	0	0	0	0	0
82	3	37	13	7120	15				3	2	1	1	0	1
83	1	41	8	1603	9	16	1		4	3	0	3	0	3
84	3	40	9	3407	14				5	0	0	0	0	0
85	3	42	10	2713	11				4	0	0	0	0	0
86	1	39	8	2318	17	18	1		4	3	0	3	0	3
87	2	39	15	1091	10				2	1	0	1	0	1
88	2	42	11	1810	17				3	0	0	0	0	0
89	2	43	12	3165	10				3	1	1	0	0	0
90	2	38	14	3573	14				4	0	0	0	0	0
91	2	39	14	1407	13				3	0	0	0	0	0
92	2	43	11	678	4				2	0	0	0	0	0
93	2	40	15	1742	13				3	2	0	2	2	0
94	3	37	10	1789	11				4	0	0	0	0	0
95	3	39	12	2168	5				2	0	0	0	0	0
96	1	41	9	2769	24	32	4		5	0	0	0	0	0
97	3	38	11	4774	16				4	0	0	0	0	0
98	1	38	na	na	na	na	na		2	2	0	2	2	0
99	3	38	12	2922	8				4	0	0	0	0	0
100	1	39	9	4897	27	35	1		2	0	0	0	0	0
101	2	39	10	1379	7				3	0	0	0	0	0
102	2	35	12	1479	7				3	2	1	1	0	1
103	2	41	13	2290	12				3	0	0	0	0	0
104	2	37	12	2470	10				1	0	0	0	0	0
105	2	42	12	1126	8				3	0	0	0	0	0
106	2	41	12	1726	11				4	0	0	0	0	0
107	2	35	19	2500	7				1	0	0	0	0	0
108	2	43	13	1426	6				2	0	0	0	0	0
109	2	38	13	4073	15				3	1	0	1	0	1
110	2	37	18	563	10				3	0	0	0	0	0
111	1	41	10	871	7	7	2		3	1	0	1	0	1
112	1	36	8	3070	18	24	2		4	1	0	1	0	1
113	1	38	9	791	6	9	1		3	0	0	0	0	0
114	1	36	9	1040	12	8	1		3	2	0	2	0	2
115	1	33	10	1119	10	9	2		3	0	0	0	0	0
116	1	36	9	1963	10	7	1		4	2	0	2	0	2
117	1	36	7	2779	14	25	3		3	1	0	1	1	0

<sup>a</sup>Probes used: 1 = X, Y, 18, 13/21; 2 = X, Y, 13, 18, 21; 3 = X, Y, 13, 16, 18, 21; 4 = X, Y, 13, 14, 15, 16, 18, 21, 22.  
stim. = stimulation; IVF = in-vitro fertilization; Spont. = spontaneous; na = not available.