

## Article

# Wide range of chromosome abnormalities in the embryos of young egg donors



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Santiago Munné has been director of PGD at Reprogenetics since 2001. This company, which he founded, offers PGD services to over 150 IVF centers in the US, and also has labs in Spain and Japan. Originally from Barcelona, Spain, Dr Munné gained his PhD in genetics from the University of Pittsburgh and joined Dr Jacques Cohen at Cornell University Medical College, New York in 1991. There he developed the first PGD test to detect embryonic numerical chromosome abnormalities. His work has been recognized by several prizes: in 1994, 1995, 1998 and 2005 from the Society for Assisted Reproductive Technology, and in 1996 from the American Society for Reproductive Medicine. Recently the PGD team has shown higher pregnancy rates and lower spontaneous rates in women of advanced age undergoing PGD. This team has performed more than 700 PGD cycles for translocations and over 5500 PGD cycles for chromosome abnormalities related to advanced maternal age or with recurrent pregnancy loss. Dr Munné has more than 150 publications to his name, and is a frequent lecturer, both nationally and internationally, on his team's work and the field of preimplantation genetics. He was recently blessed with his first daughter, Mar.

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## Abstract

Embryo chromosome studies show high rates of abnormalities, above 50%, but most embryos studied were from patients aged 35 and older. The objectives of this study were firstly, to evaluate the rate of chromosome abnormalities in embryos from young egg donors, and secondly, to compare the range of chromosome abnormality rates between donors and non-egg donor cycles, both undergoing preimplantation genetic diagnosis (PGD) for infertility using fluorescence in-situ hybridization analysis with probes for chromosomes X, Y, 13, 15, 16, 18, 21, and 22. On average, only 43% of the embryos were chromosomally normal, while the comparison group had euploidy rates between 34 (age group 18–34) ( $P < 0.001$ ) and 21% (age group 40–45) ( $P < 0.001$ ). There was considerable variation between donor cycles, with almost one-third having less than 30% normal embryos. Also, within donors and recipients repeating several IVF cycles with PGD, only 29–56% of the second PGD cycles had similar rates of normal embryos to the first cycle, while in the comparison group it was 64%. The results can explain why some egg donors are successful whereas others are not, and may also show that a policy of PGD for first time egg donors is appropriate and indicated.

**Keywords:** aneuploidy, chromosome abnormalities, egg donation, oocyte donation, PGD

## Introduction

Preimplantation genetic diagnosis (PGD) is now offered routinely in many fertility centres in order to improve the outcome of IVF. The rationale of PGD during an IVF cycle is that the majority of embryos in couples 35 and older are chromosomally abnormal (Munné *et al.*, 1995; Márquez *et al.*, 2000; Magli *et al.*, 2001; Bielanska *et al.*, 2002), and these abnormalities are usually incompatible with implantation, so the IVF outcome should improve by selecting those that are euploid (Munné *et al.*, 1993). Indeed, several controlled studies have shown that implantation rates, spontaneous abortions and take-home baby rates improve after PGD (Gianaroli *et al.*, 1999; Munné *et al.*, 1999, 2003). This improvement, however, occurs only when a single cell is biopsied from day 3 embryos. A recent prospectively randomized study demonstrated that

biopsying two cells per embryo was detrimental to embryo viability (Staessen *et al.*, 2004; Cohen and Munné, 2005).

The indications for PGD during an IVF cycle are multiple. The most common is (i) advanced maternal age, defined loosely as 35 and older, but applied mostly to patients 38 and older; followed by (ii) repeated implantation failure (Gianaroli *et al.*, 1999, 2001, 2003; Kahraman *et al.*, 2000; Pehlivan *et al.*, 2002; Wilton *et al.*, 2003), and (iii) recurrent pregnancy loss (Vidal *et al.*, 1998; Pellicer *et al.*, 1999; Rubio *et al.*, 2003; Munné *et al.*, 2005; Platteau *et al.*, 2005), (iv) previous trisomic conception (Munné *et al.*, 2004b), and (v) male factor (Silber *et al.*, 2003).

Young egg donors are traditionally assumed to have low rates of chromosome abnormalities, and young donors less than infertile

patients, even those with comparable ages. Some patients undergoing oocyte donation cycles have recently requested or been offered PGD. There are several reasons for this. Firstly, many recipients have previous experience with PGD, either because they used it in a previous cycle or because they have been made aware of studies in young patients where there can be a 40% rate of chromosome abnormalities in embryos (Munné *et al.*, 1995, Márquez *et al.*, 2000). Secondly, the 10–15% extra cost of PGD is acceptable to maximize the odds of pregnancy in the egg donor cycle. Thirdly, donor-cycle patients are going to great lengths to achieve pregnancy and wish to minimize the risk of a trisomic conception even if the donor is young.

The only study so far on chromosome abnormalities in the embryos of egg donors is by Reis Soares *et al.* (2003), in which they compared the rate of chromosome abnormalities in egg donor embryos with those from patients undergoing PGD for X-linked diseases. They found a significant difference in oocytes retrieved, 25 and 15, in chromosome abnormalities, 56 and 37% ( $P < 0.01$ ), and implanted less, 25–36.4% respectively. They concluded that larger cohorts of embryos could show higher rates of chromosome abnormalities.

Thus, the objectives of this study were: to evaluate the rate of chromosome abnormalities in young egg donors to validate the scant data available; to determine whether certain oocyte donors produce significantly more chromosome abnormalities than others; to examine the ranges of chromosome abnormalities between and within donors; and to compare the data with a comparable age group of infertile patients.

## Materials and methods

### Study and comparison subjects

Patients ( $n = 114$ ) had their egg donor cycles ( $n = 124$  cycles), embryo biopsy and cell fixation at either the Reproductive Specialty Medical Centre, Newport Beach, CA, USA ( $n = 63$  cycles), or at Zouves Fertility Centre, Daly City, CA, USA ( $n = 28$ ), or at 12 other IVF centres ( $n = 33$ ).

In order to determine the baseline of chromosome abnormalities found in the general IVF population, the study included 522 PGD cycles from 398 patients who did not have egg donation and who were treated at the same two IVF centres providing the majority of egg donor PGD cycles, that is, the Reproductive Specialty Medical Centre ( $n = 107$ ), and at Zouves Fertility Centre ( $n = 415$ ). The indications for PGD in these patients were various: advanced maternal age ( $n = 309$  cycles); requested by the patient ( $n = 140$ ); previous trisomic conception and/or spontaneous pregnancy loss ( $n = 27$ ); male factor ( $n = 23$ ); or repeated implantation failure ( $n = 23$ ).

With the exception of cycles performed at Zouves Fertility Centre, for the majority of the other PGD procedures biopsy and fixation were performed by the mobile IVF team of embryologists of IVF laboratories, LLC (Encino, CA, USA).

Informed consent for PGD was obtained from all patients, and each was followed up as part of quality control. According to the Western Institutional Review Board (WIRB), Olympia, WA, USA, this study was exempt from IRB approval because the

Common Rule regulation 45 CFR 46.101(b)(4) states in part: ‘... research, involving the collection or study of existing data, documents, records, pathological specimens, if these sources are publicly available or if the information is recorded by the investigator in such manner that subjects cannot be identified, directly or through identifiers linked to subjects’.

### Embryo biopsy, fixation, and FISH analysis

Embryo biopsy and cell fixation were performed at the IVF centres; while cells, once fixed, were sent by courier for fluorescence in-situ hybridization (FISH) analysis (Reprogenetics LLC, NJ and CA, USA). With rare exceptions, one cell per embryo was biopsied on day 3 of development, either by zona drilling using acidified Tyrode’s solution, or laser ablation as described elsewhere (Munné *et al.*, 2003). Blastomeres were fixed individually following a protocol to minimize signal overlap and loss of micronuclei (Velilla *et al.*, 2002).

PGD analysis was performed by FISH using probes specific for nine chromosome types X, Y, 13, 15, 16, 17, 18, 21 and 22. These probes were used because in the past their use has shown improvements in implantation rates after PGD (Gianaroli *et al.*, 1999; Munné *et al.*, 2003). The FISH analysis consisted of two consecutive hybridizations following previously published protocols (Munné *et al.*, 1998). The first hybridization was performed with probes for chromosomes 13, 16, 18, 21 and 22 (Multivision PB; Vysis, Downer’s Grove, IL, USA). The second hybridization consisted of a home-made combination of probes for chromosomes X, Y, 15 and 17 (Munné *et al.*, 1998). Scoring was performed by eye without the need of any software.

If the specific signals for a chromosome were not clearly diagnosable, a third hybridization using a probe binding to a different locus for that chromosome was used (Colls *et al.*, 2004).

A previously described scoring criterion was used for differentiating between (i) close signals from two homologous chromosomes and (ii) two domains belonging to a split signal of a single chromosome (Munné and Weier, 1996).

Some apparently chromosomally abnormal embryos could not be fully reanalysed due to time constraints in the IVF laboratories. The guidelines for chromosomal classification of single cells as described previously (Munné *et al.*, 2004a) were followed, and are: (i) when the cell had two copies of each chromosomes the embryo was classified as normal; (ii) when the cell had three or more copies of each of the nine chromosomes the embryo was classified as polyploid; (iii) when the cell had one or fewer copies of each chromosome, the embryo was classified as haploid; (iv) when the cell had one or two chromosomes with an abnormal number of copies, the embryo was classified as aneuploid, and (v) when the cell had three or more chromosomes with an abnormal number of copies but the cell was not haploid or polyploid, the embryo was classified as complex abnormal.

Some donors and some recipients underwent more than one PGD cycle. To compare these cycles, a past definition

of 'predictability' was used, defined as having a similar rate ( $\pm 20\%$ ) of euploid embryos in the first and successive cycles (Munné *et al.*, 2004b).

## Statistical analysis

The purpose of this paper was to compare the incidence of chromosomal abnormality between patients using young egg donors, supposedly fertile, and patients undergoing IVF cycles after experiencing fertility problems. Under these circumstances, it is clearly most important to disentangle the age effect, which is known to influence chromosome abnormalities, and the effect of group membership. As expected, the IVF groups were a great deal older on average than the egg donor group, all of whom were under 35 years of age, so that for analytical purposes three age comparison IVF groups were generated, being: up to 34, and 35–39, and 40 and older. Since some patients had multiple cycles, the data for each patient were pooled so that a patient was represented only once in the study.

The proportions of normal embryos, those with aneuploidy, and those with other abnormalities, were analysed in a 2 (age group)  $\times$  2 (patient group) factorial structure. Since the variables were proportions, logistic regression was used, but the summaries will be presented throughout as proportions.

To analyse the variation of chromosome abnormality rates between repeated cycles, it was necessary to overcome the problem that the dispersion in binomial variation is partly dependent on the level of incidence. The observed variance was calculated in repeat cycles, and divided by the theoretical binomial variation. One would therefore expect this ratio to be, on average, around 1.0. Values greater than 1.0 would indicate over-dispersion.

## Results

A total of 114 patients underwent 124 donor cycles with PGD. The average age of the recipients was 40.8, ranging from 31 to 50, and that of the donors was 25.6, ranging from 18 to 35.

## Chromosome abnormalities

Overall, 1839 embryos were biopsied from the 124 patient egg donor cycles (average 14.7 per cycle), of which 64 did not have a diagnosis, generally because the fixed cell had no nucleus or was not properly fixed. Of the remaining 1775, 754 (43%) were chromosomally normal for the chromosomes analysed, 444 (25%) were aneuploid, and 562 (32%) had other abnormalities. **Table 1** shows these results per patient, not per embryo.

The comparison group was divided into three maternal age groups, 18–34 with 94 patients (group B), 35–39 with 168 patients (group C), and 40–45 with 153 patients (group D). The total of patients in these three groups adds up to 415 instead of 398; this is because 17 patients had two cycles, in one of which they were in one age group, but in another, an older age group.

The frequencies of chromosome abnormalities in these three groups are summarized in **Table 1**. Group 18–34 had 34%

normal embryos, group 35–39 had 30%, and 40–45 group had 21%. The age group most comparable with the egg donor group was 18–34; but still the average age was 31.6 compared with 25.4 for the egg donor group. Thus it is not surprising that there were statistically significant differences in the rates of chromosome abnormalities between the egg donor group and all other groups ( $P < 0.001$ ) (**Table 1**).

## Distribution of abnormalities within groups

**Table 2** shows the distribution of chromosome abnormalities within groups. Within each group, there was a surprising variation between cycles. For instance, while the bulk of the egg donor group had between 31–60% normal embryos, 27% of their cycles had only 0–30% normal embryos; results more often found in patients over 40 rather than under 35.

Interestingly, the distribution of chromosome abnormalities was similar between egg donors and the 18–34 group, and had a typical bell shape, while the 35–39 and even more the 40–45 group, had curves biased to the left (**Figure 1**) with more internal variation.

## Distribution of abnormalities in repeat cycles

**Table 3** shows the recipients and donors who underwent more than one PGD cycle. Nine recipients and 14 donors had two or more donor cycles, but no recipient or donor had two or more cycles with the same donor or recipient. In total, 56% (5/9) of recipients and 29% (4/14) of donors had similar results (see definition of predictability above) between cycles. There were 91 comparison group patients with two or more PGD cycles, and of those 58 (64%) had similar results between cycles. Due to the small sample numbers, their differences were not statistically significant.

The statistical analysis showed that the dispersion for the egg donor group ( $1.88 \pm 0.88$ ), was still not significantly larger than any of the comparison groups ( $<35$  was  $0.87 \pm 0.27$ ,  $35-40$  was  $0.69 \pm 0.16$ , and  $>40$  was  $0.87 \pm 0.18$ ). However, when analysing each egg donor, one of them had a dispersion of 7.46, with the first cycle having 12.5% normal embryos (1/8) and the second 75% (12/16), which was significantly different from a 1.0 dispersion ( $P < 0.01$ ).

## Pregnancy outcome

**Table 4** shows the pregnancy outcome of the four PGD groups. There was no difference in the pregnancy rate per transfer observed in the egg donor group and the 18–34 comparison group, but those two had a significantly higher pregnancy rate than the 35–39 ( $P < 0.05$ ) and the 40–45 comparison groups ( $P < 0.001$ ).

**Table 1.** Chromosome abnormalities per group.

Group <sup>a</sup>	Mean age (years)	Embryos (n)	Patients (n) <sup>b</sup>	Frequency <sup>c</sup> of embryo type ( $\pm$ SD)			Mean no. of normal embryos/cycle
				Normal	Aneuploid	Other	
A	25.4	1775	114	0.43 $\pm$ 0.02	0.25 $\pm$ 0.01	0.32 $\pm$ 0.02	6.0
B	31.6	1062	94	0.34 $\pm$ 0.02	0.32 $\pm$ 0.01	0.34 $\pm$ 0.02	3.7
C	37.4	1975	168	0.30 $\pm$ 0.01	0.33 $\pm$ 0.02	0.37 $\pm$ 0.01	3.2
D	41.5	1656	153	0.21 $\pm$ 0.01	0.35 $\pm$ 0.02	0.43 $\pm$ 0.02	1.8

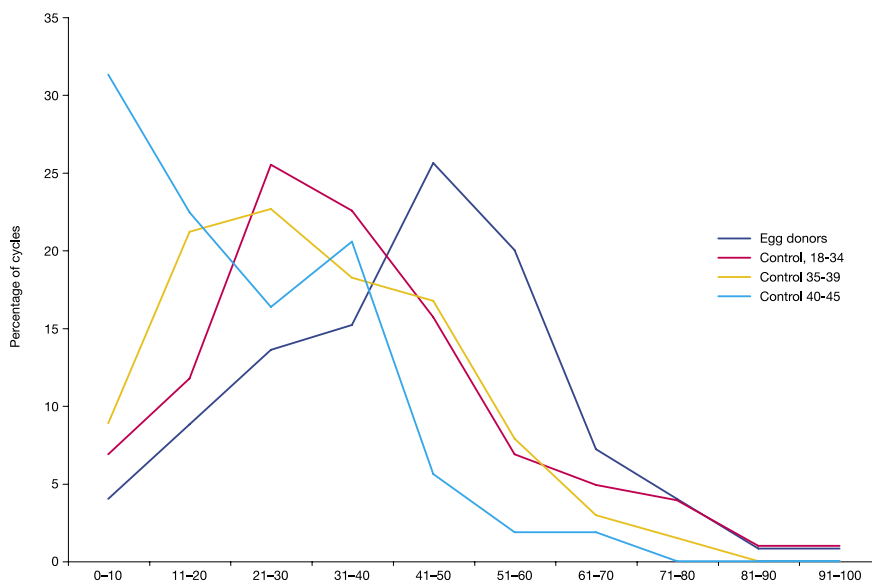
<sup>a</sup>Group A = egg donors age 18–35; infertile age groups B = 18–34 years, C = 35–39 years, D = 40–45 years.

<sup>b</sup>Total of patients is 415 and not 398 because 17 patients had two cycles, each cycle in different age groups.

<sup>c</sup>Significant differences in frequency of normal embryos: A > B, A > C, A > D, B > D, C > D –  $P < 0.001$ ; B > C –  $P < 0.05$ .

**Table 2.** Variation in chromosome abnormalities within donors and age groups. Values are number of cycles.

% normal	Egg donors	Infertile comparison group		
		18–34 years	35–39 years	40–45 years
0–10	5	7	18	67
11–20	11	12	43	48
21–30	17	26	46	35
31–40	19	23	37	44
41–50	32	16	34	12
51–60	25	7	16	4
61–70	9	5	6	4
71–80	5	4	3	0
81–90	1	1	0	0
91–100	1	1	0	0
Total	125	102	203	214



**Figure 1.** Variation in chromosome abnormalities within donor and age groups.

**Table 3.** Frequency of normal embryos in cycles involving the same recipient or donor.

<i>Donor</i>	<i>Recipient couple</i>	<i>No. of cycles</i>	<i>% normal range</i>	<i>Predictability<sup>a</sup></i>
Multiple	1	2	17–54	No
Multiple	2	2	44–50	Yes
Multiple	3	2	33–38	Yes
Multiple	4	2	43–45	Yes
Multiple	5	2	0–24	No
Multiple	6	2	13–75	No
Multiple	7	2	55–59	Yes
Multiple	8	2	25–42	Yes
Multiple	9	3	25–73	No
1	Multiple	2	44–67	No
2	Multiple	2	25–42	Yes
3	Multiple	6	43–65	No
4	Multiple	2	40–50	Yes
5	Multiple	2	38–50	No
6	Multiple	3	27–50	No
7	Multiple	2	40–75	No
8	Multiple	2	17–41	No
9	Multiple	2	29–83	No
10	Multiple	3	23–29	Yes
11	Multiple	2	54–73	No
12	Multiple	2	33–33	Yes
13	Multiple	2	25–50	No
14	Multiple	2	25–48	No

<sup>a</sup>Predictability: 20% or less variation between cycles (Munné *et al.*, 2004b).

**Table 4.** Pregnancy outcome across donors and age groups. Group A, egg donors age 18–35; infertile age groups B = 18–34 years, C = 35–39 years, D = 40–45 years.

<i>Group (donor or infertile)</i>	<i>Mean age (years)</i>	<i>No. cycles</i>	<i>Informative</i>	<i>Unknown<sup>m</sup></i>	<i>Transferred</i>	<i>Pregnancies</i>	<i>% pregnancies/oocyte retrieval</i>	<i>Mean no. embryos replaced</i>	<i>% implanted</i>
A	25.5	124	121	3	113/121	61	50 <sup>a</sup>	2.1	39.4 <sup>f</sup>
B	31.6	103	103	0	99/103	52	50 <sup>d</sup>	2.8	33.0 <sup>h,j</sup>
C	37.4	204	204	0	187/204	81	40 <sup>b,d</sup>	2.7	24.1 <sup>g,i,j</sup>
D	41.5	216	216	0	174/216	50	23 <sup>c,e</sup>	2.4	12.0 <sup>g,k</sup>

<sup>a-k</sup>Significant differences were found in the following comparisons: a vs b –  $P < 0.05$ ; h vs i –  $P < 0.01$ ; a vs c, d vs e, f vs g, j vs k –  $P < 0.001$ .

<sup>m</sup>Lost to follow-up or no data available yet.

## Discussion

The present study indicates that young donors, presumably fertile, produce high rates of chromosomally abnormal embryos (57%). Nevertheless, because they produce many oocytes, there are enough chromosomally normal embryos to result in high pregnancy rates after IVF. The findings of this study suggest an unexpectedly wide range of chromosome abnormality rates between donors, but similar rates and variations are found in younger infertility patients.

The comparison group of infertile patients, 18–34 years old, undergoing PGD, produced 66% chromosomally abnormal embryos, higher than the egg donor group. However, considering that this comparison group was 5 years older, on average, than the egg donor group, one can assume very similar rates of chromosome abnormalities for the same age. This, if true, would mean that infertility in young couples (<35) may be attributed to other factors, and not to chromosome abnormalities in oocytes. One may also consider that it is certain aspects of follicular stimulation that could possibly cause aneuploidy in both donor and patients eggs. Whatever the reason, true controls such as analyses of eggs from donor and patient embryos in natural cycles are not available.

The only other study concerning egg donors is the one by Reis Soares *et al.* (2003), in which they compared chromosome abnormality rates between two groups of fertile patients, that is, egg donors and X-linked disease patients. In that study, they found higher numbers of eggs produced but also more chromosome abnormalities in the egg donor group. They suggested that a higher ovarian response (and/or stimulation) caused an increase in chromosome abnormalities. In the present study, the egg donor group produced on average 14.6 biopsied embryos, compared with 11.5 from the 18–34 years old comparison group. Although this is an imperfect measure of the egg cohort size, it supports the findings of Reis Soares *et al.* (2003) in that perhaps if the results had been compared with another fertile group, differences would have been found from the egg donor group. Certainly, a study with four arms (fertile egg donor, fertile X-linked, infertile and natural cycle) is needed.

Thus, the large oocyte cohort size from apparently normal egg donors, produced by robust hormonal stimulation, appears to have rates of chromosome abnormalities of above 50%, just like those of infertile patients of similar age. It is therefore proposed that PGD may be warranted in this group of patients and donors.

Another interesting finding of this study is the great variability in chromosome abnormality rates between egg donors. While a majority had more than 30% euploid embryos, 27% had less than that, a value more normally found in infertile patients of 40 years and older.

This variability is not exclusive to egg donors. Comparison between the 18–34-year group and the egg donor group showed they had very similar distribution curves (**Figure 1**), again confirming that these two groups of patients are similar. The variability in chromosome abnormality rates is even more accentuated in older patients, showing that maternal age is only a gross indicator of

chromosome abnormalities in human IVF embryos.

This variation cannot be attributed to the fact that a single cell has been biopsied, since in the past an error rate of only 6% has been documented (Munné *et al.*, 1998). Also unlikely is the possibility that cells allocated to a specific future tissue (Hansis and Edwards, 2003; Edwards and Hansis, 2005), would have different chromosome abnormalities affecting the results. Since these cells can only be identified with tissue-specific markers and those were not used during biopsy, the embryos were biopsied at random and any variations due to cell allocation should even out. Furthermore, studies on blastocyst chromosome analysis do not show differences in chromosome abnormalities between the inner cell mass and the trophectoderm (Evsikov and Verlinsky *et al.*, 1998), and therefore one would not expect them earlier on.

To avoid using an egg donor that had high chromosome abnormalities two or more times, PGD might be indicated for their first donation, on the basis of 57% chromosome abnormalities. This presupposes that PGD results in a first cycle are repeated in a second PGD cycle. Two studies have analysed the predictability of PGD results and both found that the first PGD cycle results are generally indicative of future PGD cycles (Ferraretti *et al.*, 2004; Munné *et al.*, 2004b). Predictability was defined as having similar rate ( $\pm 20\%$ ) of euploid embryos in the first and successive PGD cycles, and was found in only 66% of couples, probably because of changes in hormonal stimulation regimes from one cycle to the next (Munné *et al.*, 2004b). In the present study, the predictability of the comparison cycles (64%) was similar to that of the previous study, 66%; but for the egg donor cycles, predictability was only 56% for recipients and 29% for egg donors. These differences actually might change significantly with a larger sample, and might represent variability induced by the fact that only one of the partners in the couple is the same between cycles. It would be interesting to determine, with a larger sample, if the male or the female partner provides more predictability to successive PGD results. Although intuitively most aneuploidies are maternal in origin, in egg donors 32% of embryos were detected with chromosome abnormalities other than aneuploidy (polyploid, haploids, complex abnormal). These abnormalities are increased in extreme male factors (Silber *et al.*, 2003), and thus both partners may have an impact on the normality of the embryo. It may be possible that the reason for suggesting an egg donor is not always maternal age driven. In such patients one could consider a sibling oocyte study using a sperm donor in the absence of oligoasthenoteratospermia.

Pregnancy and implantation rates in all PGD groups declined with maternal age (**Table 1**), probably because the average of normal embryos per cycle was six and four in the donor and 18–34 age groups, respectively, compared with only two in the 40 and older. Thus, even if the embryos were normal for the chromosomes tested, morphological selection could also be applied in the young groups but not in the older one.

The same reasoning applies to comparison of the donor and 18–34 age groups. Both had similar pregnancy and implantation rates although the 18–34 comparison group had significantly more chromosome abnormalities than the donor group ( $P < 0.05$ ). The reason for having similar pregnancy rates might be that in both groups there were enough normal embryos (six and four respectively) to do genetic and morphological selection to

replace on average 2.4–2.7 embryos (Table 4).

In summary, while egg donors had lower rates of chromosome abnormalities than a comparison group of infertile patients, the difference could be attributed to slightly higher maternal ages in the comparison group. Thus fertile donors might actually have rates of abnormalities similar to infertile patients of the same age. In any case, the range of chromosome abnormalities found in young donor embryos was higher than suspected, from 0 to 100%, which shows that success rates of egg donation are considerably limited because of poor morphological selection power without PGD; therefore, a policy to perform PGD in the first cycle of egg donors may well be appropriate and indicated.

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