

Article

Preimplantation testing for chromosomal disorders improves reproductive outcome of poor-prognosis patients



Dr Anver Kuliev received his PhD in Clinical Cytogenetics from Moscow Research Institute of Human Morphology in 1969. In 1979 he took the responsibility for the World Health Organization (WHO)'s Hereditary Diseases Program in Geneva, where he developed community-based programmes for prevention of genetic disorders and early approaches for prenatal diagnosis. He moved to the Reproductive Genetics Institute in 1990, where he heads the WHO Collaborating Center for Prevention of Genetic Disorders, and scientific research in prenatal and preimplantation genetics. He is an author on more than a hundred papers and nine books in the above areas, including three books in the field of Preimplantation Genetics.

Dr Anver Kuliev

Y Verlinsky, I Tur-Kaspa, J Cieslak, A Bernal, R Morris, M Taranissi, B Kaplan, A Kuliev¹
Reproductive Genetics Institute, 2825 N Halsted St, Chicago IL, 60657
¹Correspondence: Tel: +773 472-4900; Fax: +773 871-5221

Abstract

The clinical impact of PGD was evaluated through the analysis of the reproductive outcome before and after PGD in the same group of poor prognosis IVF patients, undergoing PGD for chromosomal abnormalities. Based on a series of 2359 PGD cycles, resulting in the establishment of 498 chromosomal abnormality-free clinical pregnancies, the reproductive history prior to PGD was analysed. Of 483 previous pregnancies analysed in patients with 432 pregnancies generated after PGD for aneuploidies, 328 (68%) ended in spontaneous abortions, in contrast to 28.4% after PGD, with only 155 (32%) resulting in deliveries, compared with 71.9% take-home baby rates after PGD. The patients experienced 315 previous IVF attempts, resulting in the transfer of 706 embryos in 308 cycles, of which only 49 (6.9%) implanted, compared with a 34.9% implantation rate observed in the same patients after PGD. Similar analysis of the previous reproductive outcomes of 45 carriers of balanced translocations achieving pregnancies following PGD, showed even stronger clinical impact, with a reduction of spontaneous abortions from 87.8% to 17.8%, and improvement of take-home baby rate from 11.5% to 81.4% after PGD. The results demonstrate a strong clinical impact of PGD, resulting in improvement of implantation rate, reduction of spontaneous abortions and increase in the take-home baby rate.

Keywords: chromosomal disorders, FISH, implantation rate, preimplantation genetic diagnosis, spontaneous abortion rate, take-home baby rate

Introduction

Because half of the oocytes and embryos in poor prognosis IVF patients are chromosomally abnormal, there is no doubt as to the potential utility of preimplantation genetic diagnosis (PGD) for improving IVF success rates, although there are still not sufficient data to quantify the expected clinical outcome. Previous reports presented the evidence for the positive clinical outcome after PGD in terms of the improved implantation and decreased spontaneous abortion rates, which should have contributed to the improvement of live birth rate as well, although this was not yet documented (Gianaroli *et al.*, 1999; Munné *et al.*, 1999; 2003). In these studies the clinical outcome of PGD cycles were compared with that of the matched controls, suggesting a higher implantation rate, despite transferring

fewer embryos in the PGD group. The implantation rate almost doubled in couples with fewer than two failed cycles and with more than eight zygotes available for testing (Munné *et al.*, 2003).

Although prospective randomized studies would have been the most appropriate approach to quantify the clinical impact of PGD (Gianaroli *et al.*, 1999), these have not yet been successful, because, if informed, the poor prognosis patients usually prefer to undergo PGD rather than being randomized. Also, due to the relative novelty of PGD, the accumulated experience of PGD for chromosomal disorders has still been limited in most centres, with only a few centres having performed a sufficient number of PGD cycles, to evaluate PGD impact on the take-home baby rate (Verlinsky *et al.*, 2004). On the other hand, this information

is of growing practical relevance, as the patients undergoing the assisted reproduction require accurate information about the actual benefit of PGD in terms of the improvement of the take-home baby rate, in order to make their own choice regarding the use of PGD.

Significant improvement of the reproductive outcome after PGD in comparison with the previous obstetric history of the same patients prior to undergoing PGD has recently been reported in a series of 193 patients (Gianaroli *et al.*, 2004). The present paper analyses the reproductive outcome of one of the largest world experiences of 433 patients prior to undergoing PGD, demonstrating considerable improvement of implantation rate, together with a reduction of spontaneous abortions and increase of take-home baby rate after PGD for chromosomal disorders.

Materials and methods

During a 10-year period, we performed the world's two largest PGD series of 2176 cycles for aneuploidies for 1493 poor prognosis IVF patients, including poor responders, those of advanced reproductive age, repeated spontaneous abortions and previous IVF failures, and 183 PGD cycles for 130 patients with balanced chromosomal translocations (**Table 1**).

Of 442 pregnancies, resulting from PGD for aneuploidies, previous reproductive history was available for 432 pregnancies, generated from 388 patients of average age 37 years, allowing the analysis of the implantation, spontaneous abortion and live-birth rates in the same patients before and after PGD.

Of 183 cycles performed for PGD of translocations, unbalanced translocation-free pregnancies were obtained in 45 patients (average age 32.5 years). According to their previous reproductive history, six patients had never been pregnant, and 39 had had 135 spontaneous pregnancies, the outcomes of which were analysed in comparison to the same parameters after PGD.

Implantation rate was evaluated as the presence of fetal heartbeat at 6 weeks of pregnancy, while ectopic pregnancies and terminations of pregnancies were excluded from the analysis.

PGD was performed either by analysis of the first and second polar bodies (PB1 and PB2), removed simultaneously following fertilization on day 1, or by single blastomere analysis, biopsied on day 3, or by both PB1, PB2 and blastomere analysis. The latter is currently the main strategy, allowing the detection of both meiotic and mitotic errors. Three to nine chromosomes (13, 15, 16, 17, 18, 21, 22, X and Y) were tested as described elsewhere (Kuliev *et al.*, 2003; Verlinsky and Kuliev, 2004). The methods for PGD of translocations are also described elsewhere, including PGD for reciprocal and Robertsonian translocations (Verlinsky *et al.*, 2002; Verlinsky and Kuliev, 2004). In addition to locus-specific identifier, centromeric enumeration and telomeric probes, the method of blastomere nuclear conversion into metaphase chromosomes was used utilizing whole chromosome paints (Verlinsky and Kuliev, 2004).

Embryo transfer was routinely performed on day 3 or 5, with only 1–2 blastocysts being transferred at the present time (the mean number of embryos transferred was 2.2 in PGD for aneuploidies, and 1.7 in PGD for translocations). Based on the above testing, the embryos without chromosomal abnormalities were transferred back to patients, while the abnormal embryos were further tested to confirm the diagnosis, according to the signed informed consent, approved by Institutional Review Board.

Chi-squared analysis and the programme for epidemiologists (PEPI, 2005) were used for statistical analysis and to calculate odds ratio at 95% confidence interval.

Table 1. Reproductive outcome of 2359 preimplantation genetic diagnosis cycles performed for aneuploidies and translocations. Values are numbers unless otherwise stated.

	<i>Aneuploidies</i>	<i>Translocations</i>	<i>Total</i>
Patients/cycles	1493/2176	130/183	1623/2359
Embryos with FISH results	8213	1440	9653
Normal embryos transferred	3880 (2.2 per transfer)	215 (1.7 per transfer)	4095
No. transfers	1744	126	1870
Pregnancies (%)	453 (26.0)	45 (35.7)	498 (26.6)
Implantation rate (%)	14.7	24.7	N/A
Abortions (%)	122 (27.9) ^a	8 (18.6) ^b	130 (27.5)
Ongoing pregnancies (or unknown)	16	2	18
Children born	376 (of 308 deliveries)	40 (of 35 deliveries)	416 (of 343 deliveries)
Mean maternal age (years)	38.5	33.2	N/A
Take-home baby rate per pregnant patient (%)	71.6 ^a	81.4 ^b	N/A

FISH: fluorescence in-situ hybridization.

^aBased on 430 pregnancies with known outcomes, i.e. excluding seven ectopic pregnancies and 16 with unknown outcomes.

^bBased on 43 pregnancies with known outcomes (two pregnancies were ongoing).

Results

PGD for aneuploidy

Testing for aneuploidies in 2216 PGD cycles resulted in detection and transfer of 3880 aneuploidy free embryos from 8213 with fluorescence in-situ hybridization (FISH) results, yielding 453 (26%) clinical pregnancies in 1744 transfers (**Table 1**). As cytogenetic results of this study have been partially described elsewhere (Kuliev *et al.*, 2003; Verlinsky and Kuliev, 2004), the present analysis will concentrate on the clinical outcomes.

The data on the previous reproductive outcome were available for 432 pregnancies after PGD, generated in 388 patients. Pregnancies after PGD were obtained from transfer of 1462 embryos, resulting in 510 (34.9%) implanted sacs, 126 (28.4%) of which were lost in 121 spontaneously aborted pregnancies, 360 resulted in the birth of healthy babies in 299 pregnancies, and eight are still in progress in six ongoing pregnancies (**Figure 1**). So, overall, 299 pregnancies resulted in healthy deliveries (six ectopic pregnancies, and 10 fetal reductions were excluded from the analysis), suggesting as high as 71.9% take-home baby rate after PGD.

As seen from the previous reproductive history of this group of patients (**Figure 2**), 195 of them had undergone 315 previous IVF cycles, involving the transfer of 706 embryos, of which only 49 (6.9%) implanted. Overall, these patients, including the other 193 who had no previous IVF cycles, had 578 previous pregnancies (24 ectopic pregnancies were excluded), of which only 155 (32.1%) resulted in healthy deliveries, while the remaining 399 were unsuccessful, including 328 (67.9%) resulting in spontaneous abortions. The distribution of patients with one, two, three and four or more miscarriages is shown in

Figure 3, showing that these patients were at a disadvantage from the outset. As seen from **Figure 4**, demonstrating a comparison of the reproductive outcome before and after PGD, including the implantation rate in IVF cycles performed before undergoing PGD, PGD contributed significantly ($P < 0.001$) to implantation rate improvement from 6.9 to 34.9%, spontaneous abortion rate reduction from 67.9% to 28.4%, and take-home baby rate improvement from 32.1% to 71.9%.

PGD for translocations

The data on the previous reproductive outcome were analysed in 45 carriers of balanced translocations, who had achieved clinical pregnancies following PGD in 183 cycles from 130 patients. These pregnancies were obtained from the transfer of 86 unbalanced translocation-free embryos in 56 cycles (mean of 1.4 embryos per transfer), resulting in 53 (61.6%) implanted sacs, 9 (17.8%) of which were lost in eight spontaneously aborted pregnancies, 40 resulted in the birth of healthy babies with either normal or balanced karyotypes in 35 pregnancies, and three are still in progress in two ongoing pregnancies (**Figure 5**). Overall, 35 (81.4%) of 43 pregnancies resulted in healthy deliveries.

Six of these patients had never been pregnant, while the remaining 39 patients had 135 previous spontaneous pregnancies (**Figure 6**), of which 115 (87.8%) resulted in abortions (four ectopic pregnancies were excluded from the analysis) and only 16 (11.5%) resulted in deliveries, including the birth of four unbalanced children and one stillborn with normal karyotype. As seen from **Figure 7**, demonstrating the reproductive outcome before and after PGD for translocations, the spontaneous abortion rate was reduced from 87.8 to 17.8%, with take-home baby rate being improved from 11.5 to 81.4% after PGD.

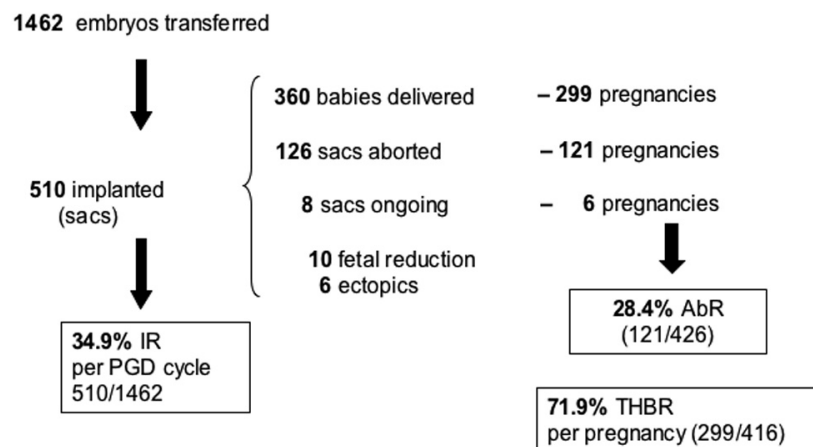


Figure 1. Previous obstetric history of 388 patients achieving 432 clinical pregnancies after PGD. ART, assisted reproduction treatment; IR, implantation rate; THBR, take-home baby rate. Mean maternal age 37.0 ± 3.3 years.

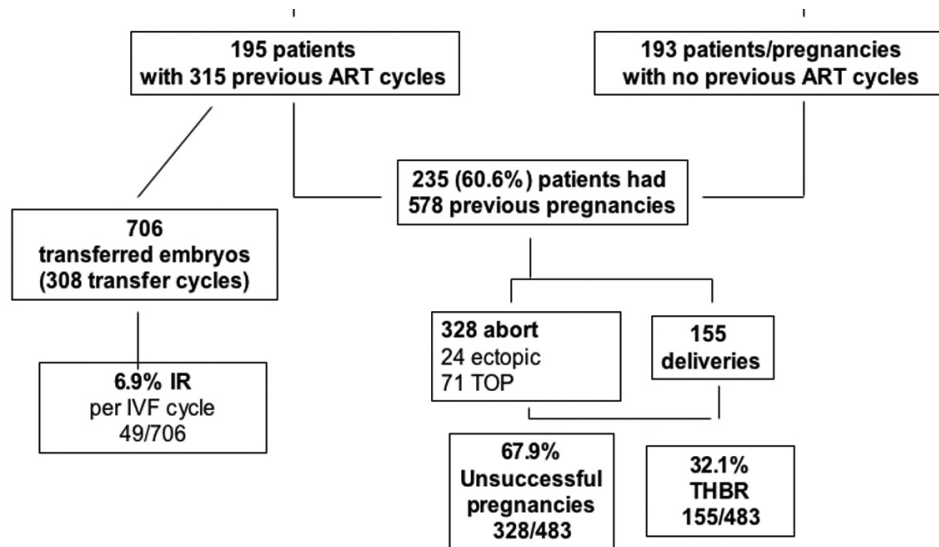


Figure 2. Previous obstetric history of 388 patients achieving 432 clinical pregnancies after preimplantation genetic diagnosis (PGD) for aneuploidies in 627 cycles. In the group with previous assisted reproduction treatment (ART) history, only a 6.9% implantation rate (IR) was achieved. In all 578 previous pregnancies observed in 235 of all patients in both groups, only 155 (32%) achieved successful deliveries, the remaining pregnancies being unsuccessful. Mean maternal age was 37 ± 3.3 years. TOP = termination of pregnancy; AbR = spontaneous abortion; THBR = take-home baby rate.

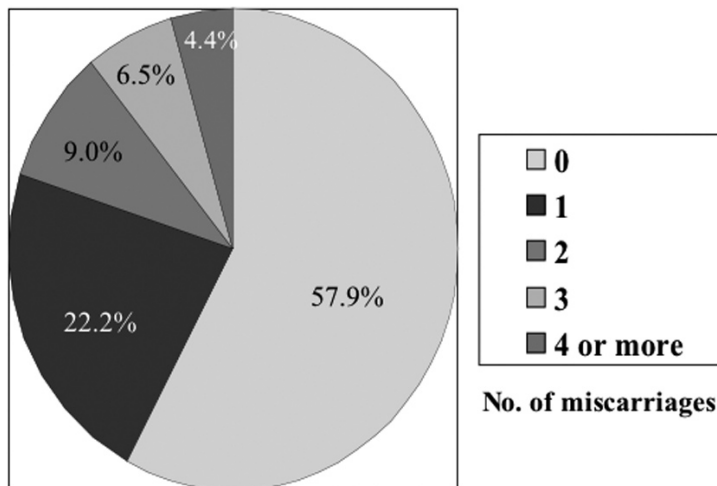


Figure 3. Distribution of previous number of pregnancy losses in patients undergoing preimplantation genetic diagnosis (PGD) for aneuploidy; of 328 abortions experienced by patients undergoing PGD prior to PGD, 22.2% had only one, 9% had two, 6.5% had three and 4.4% had four or more miscarriages; 42.1% had at least one miscarriage; 19.9% had two or more miscarriages.

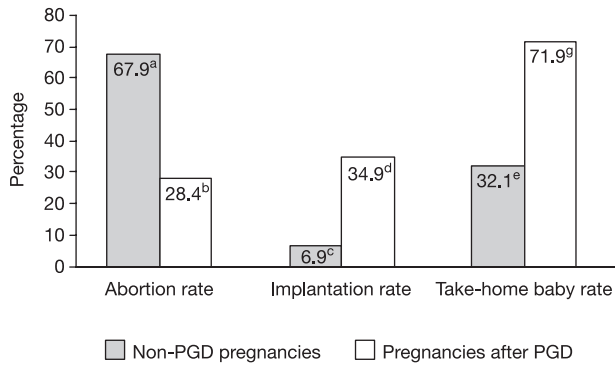


Figure 4. Outcome of pregnancies from 388 patients before and after preimplantation genetic diagnosis (PGD) for aneuploidies. Histogram representing the obstetric history in the same group of patients before and after PGD for aneuploidy. Retrospective analysis shows a significantly lower abortion rate, a significantly higher implantation rate and a greater than two-fold increase in the take-home baby rate (all $P < 0.001$). Mean maternal age was 37 ± 3.3 years; 82.1% of these PGD patients were older than 35 years. Chi-squared test: ^{a,b} $P < 0.001$; ^{c,d} $P < 0.001$; ^{e,g} $P < 0.001$.

86 embryos transferred in 56 cycles (mean 1.4)

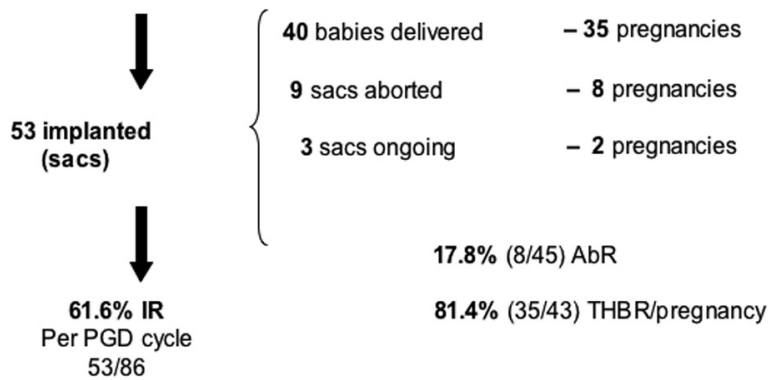


Figure 5. Implantation (IR), spontaneous abortion (AbR) and take-home baby (THBR) rates in 45 patients following preimplantation genetic diagnosis (PGD) for chromosomal translocation. Mean maternal age was 32.5 ± 3.9 years.

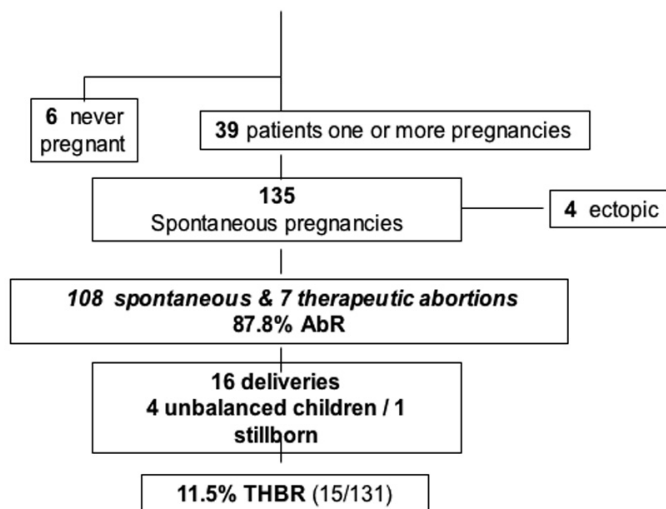


Figure 6. Previous obstetric history of 45 translocation carrier couples. Mean maternal age was 32.5 ± 3.9 years. AbR, abortion rate; THBR, take-home baby rate.

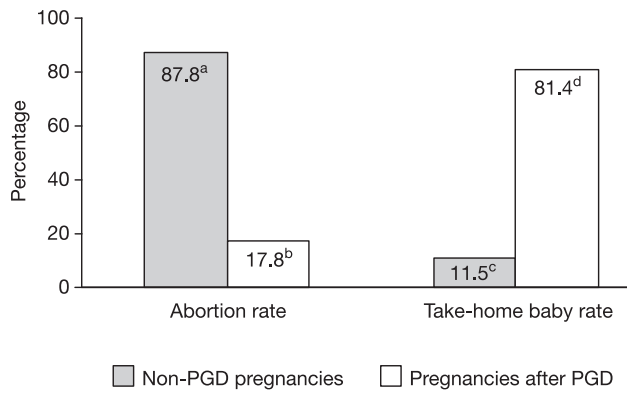


Figure 7. Outcome of pregnancies from 45 patients before and after preimplantation genetic diagnosis (PGD) for chromosomal translocation. Bar graph representing the retrospective analysis of the previous obstetric history in relation to the 45 patient/pregnancies that occurred following PGD for chromosomal translocations. A significantly lower abortion rate (17.8%) was realized along with a significantly higher take-home baby rate of 81.4% (a versus b, c versus d: both $P = 0.001$; chi-squared test). Mean age was 32.5 ± 3.9 years.

Discussion

With the current tendency for limiting the number of transferred embryos to only two, and even to one in blastocyst transfers, in the attempt to avoid the complications due to multiple pregnancies, the further improvement of IVF efficiency will be unrealistic without preselection of aneuploidy-free oocytes and embryos, as there is no reason to deliberately transfer untested embryos, more than 50% of which are chromosomally abnormal, compromising the outcome of IVF in a traditional setting. This study reports one of the world's two largest series of PGD for chromosomal disorders, providing sufficient material for analysis of the impact of PGD on live birth rate. The results presented of the reproductive outcome before and after PGD for chromosomal disorders in the same patients, support the initial observations on the positive impact of PGD on the implantation rate and pregnancy outcomes, and also an improvement of take-home baby rate (Gianaroli *et al.*, 1999; 2005; Munné *et al.*, 1999, 2003).

This is not surprising, as only one in 10 recognized pregnancies are chromosomally abnormal, in contrast to one in two in preimplantation embryos, suggesting that the majority of these chromosomally abnormal embryos are eliminated before implantation. Incidental transfer of these embryos in the absence of chromosomal testing should clearly contribute to implantation and pregnancy failures in poor prognosis IVF patients, or should affect pregnancy outcome through spontaneous abortions.

Although randomized controlled studies could still be useful to further quantify the clinical impact of preselection of chromosomally normal zygotes for embryo transfer, the results presented of the comparison of the reproductive outcome in the same group of 388 patients with and without PGD provide strong evidence for an improvement in reproductive outcome after PGD. Significant differences ($P = 0.001$) were observed for all the parameters tested, including a five-fold increase in implantation rate, more than two-fold reduction of spontaneous abortion rate and more than two-fold increase of take-home baby rate after PGD, compared with that prior to PGD. The clinical impact is even higher in PGD for translocations, with five-fold reduction of the spontaneous abortion rate, and seven-fold increase of the take-home baby rate after PGD, which is

in agreement with previous reports demonstrating considerable reduction of spontaneous abortions after PGD (Munné *et al.*, 2000; Verlinsky *et al.*, 2002; Gianaroli *et al.*, 2005).

In light of these data, the current practice of embryo selection for transfer based on morphological criteria may no longer be acceptable for poor prognosis IVF patients. In addition to an extremely high risk of establishing an affected pregnancy from the onset, this will significantly compromise the very poor chances of these patients of becoming pregnant, leaving little chance even for the implanted embryo to reach term. Although culturing embryos to day 5 (blastocyst) before transfer may to some extent allow the preselection of developmentally more competent embryos, compared with the day 3 embryo transfer, one in five aneuploid embryos may still be capable of developing to blastocyst (Magli *et al.*, 2000; Sandalinas *et al.*, 2001; Munné *et al.*, 2004). Therefore, a considerable proportion of abnormal embryos will not be eliminated even in the current shift to blastocyst transfer, but will implant and lead to spontaneous abortions, compromising the outcome of pregnancies.

PGD for chromosomal disorders has currently been carried out in over 5000 IVF cycles for poor prognosis patients, resulting in improved implantation and pregnancy rates (Munné *et al.*, 2003; Kuliev and Verlinsky, 2004; Verlinsky *et al.*, 2004; Gianaroli *et al.*, 2005). There is also increasing evidence that even sequential biopsy procedures may not affect the viability of the embryos (Cieslak *et al.*, 2005). Although further studies may be needed to further quantify the impact of PGD on the IVF efficiency, the currently available data leave no doubt of the clinical relevance of avoiding transfer of chromosomally abnormal embryos. An incidental transfer of these embryos in the absence of chromosomal testing would contribute to implantation and pregnancy failures in poor prognosis IVF patients. In addition, the majority of the chromosomally abnormal embryos that implant will further lead to spontaneous abortions and affect the pregnancy outcome. The subject of the effects of biopsy procedures on IVF outcomes will be discussed in a paper currently in preparation.

So PGD may definitively contribute to improving standards of the assisted reproduction, and may in the future replace the current practice of selection of embryos for transfer using

morphological criteria, by the preselection of chromosomally normal embryos with the highest possible potential to result in pregnancy.

References

- Cieslak J, Tur-Kaspa I, Ilkevitch Y *et al.* 2005 Developmental potential of embryos after one to three biopsy procedures for PGD. *Reproductive BioMedicine Online* **10** (suppl. 2), 17 (abstract).
- Gianaroli L, Magli MC, Ferraretti AP, Munné S 1999 Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with poor prognosis: identification of the categories for which it should be proposed. *Fertility and Sterility* **72**, 837–844.
- Gianaroli L, Magli MC, Ferraretti AP *et al.* 2004 The beneficial effects of PGD for aneuploidy support extensive clinical application. *Reproductive BioMedicine Online* **10**, 633–640.
- Kuliev A, Verlinsky Y 2004 Thirteen years' experience of preimplantation diagnosis: report of the Fifth International Symposium on Preimplantation Genetics. *Reproductive BioMedicine Online* **8**, 229–235.
- Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y 2003 Chromosomal abnormalities in a series of 6733 human oocytes in preimplantation diagnosis of age-related aneuploidies. *Reproductive BioMedicine Online* **6**, 54–59.
- Magli MC, Jones GM, Gras L *et al.* 2000 Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocysts in vitro. *Human Reproduction* **15**, 1781–1786.
- Munné S, Magli C, Cohen J *et al.* 1999 Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. *Human Reproduction* **14**, 2191–2199.
- Munné S, Sandalinas M, Escudero T 2000 Outcome of preimplantation genetic diagnosis of translocations. *Fertility and Sterility* **73**, 1209–1218.
- Munné S, Sandalinas M, Escudero T *et al.* 2003 Improved implantation after preimplantation genetic diagnosis of aneuploidy. *Reproductive BioMedicine Online* **7**, 91–97.
- Munné S, Bahce M, Sandalinas M 2004 Differences in chromosome susceptibility to aneuploidy and survival to first trimester. *Reproductive BioMedicine Online* **8**, 81–90.
- PEPI 2005 Programs for epidemiologists. Latest version available from <http://www.sagebrushpress.com/pepibook.html> [accessed 23 June 2005].
- Sandalinas M, Sadowy S, Alikani M *et al.* 2001 Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. *Human Reproduction* **16**, 1954–1958.
- Verlinsky Y, Kuliev A 2004 *Atlas of Preimplantation Genetic Diagnosis* 2nd edn. Taylor and Francis, London and New York, p. 288.
- Verlinsky Y, Cieslak J, Evsikov S *et al.*, 2002 Nuclear transfer for full karyotyping and preimplantation diagnosis of translocations. *Reproductive BioMedicine Online* **5**, 300–305.
- Verlinsky Y, Munné S, Cohen J *et al.* 2004 Over a decade of preimplantation genetic diagnosis experience – A multi-center report. *Fertility and Sterility* **82**, 292–294.

Received 24 March 2005; refereed 5 May 2005; accepted 1 June 2005.