Article

Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies

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Abstract

Preimplantation genetic diagnosis (PGD) for translocations has been shown to significantly reduce the risk of recurrent miscarriage, but because the majority of embryos produced are unbalanced, pregnancy rate is relatively low since 20% or more cycles have no normal or balanced embryos to transfer. The purpose of this study was to evaluate whether PGD could improve pregnancy outcome in translocation carriers with a history of two or more consecutive miscarriages and no live births. PGD for translocations was offered to translocation carriers with two or more previous miscarriages (average 3.5) and no live births (0/117 pregnancies) using a combination of distal and proximal probes to the breakpoints. After PGD, only 18.3% of embryos were normal or balanced. Only 5.3% of pregnancies were lost after PGD compared with 100% before PGD ($P < 0.001$). The cumulative pregnancy rate was 57.6% and the cumulative ongoing pregnancy rate was 54.5% in the short period of time of 1.24 IVF cycles, or 46.3% and 43.9% respectively per cycle. In conclusion, PGD significantly reduced losses and increased the number of viable pregnancies ($P < 0.001$). IVF plus PGD are a faster method of conceiving a live child than natural conception, at least for translocation carriers with recurrent miscarriages and no previous live births.

Keywords: *chromosome imbalance, PGD, pregnancy outcome, recurrent miscarriage, translocation*

Introduction

Carriers of reciprocal translocations have reduced fertility and are at increased risk of recurrent miscarriages and unbalanced offspring (Chandley, 1998). In males, reciprocal translocations involving a Y autosome produce azoospermia in 80% of cases and very few sperm in other cases, and those with X autosome produce 100% azoospermia (Hsu, 1994; Chandley, 1998). Translocations between autosomes are suggested to cause male infertility either by generalized pairing disruption around the genome (Burgoyne and Baker, 1984), or by XY-multivalent interactions (Chandley, 1979). Ovarian dysgenesis has also been reported to be linked to autosomal reciprocal translocations (Tupler *et al.*, 1994).

In a study including 1324 couples having experienced two or more miscarriages, 41 were identified as carriers of structural abnormalities (28 reciprocal, three Robertsonian, five pericentric inversions, four paracentric inversions, one marker chromosome). These 41 couples were followed for six years and compared with 74 of the non-carrier ones. The carriers produced 43 pregnancies, of which 30 delivered normal or balanced offspring and the others aborted, with not a single unbalanced pregnancy reaching term (Goddijn *et al.*, 2004). Thus, the unbalanced products of a translocation are usually lethal and therefore the true risk is that of pregnancy loss.

In another study on 1284 couples with two or more consecutive miscarriages, 58 (4.5%) were carriers of translocations. In the next pregnancy, the carriers of reciprocal translocations or Robertsonian translocations miscarried significantly more often, 68% and 36% respectively, than couples without structural abnormalities (28%) (Sugiura-Ogasawara *et al.*, 2004).

It has been demonstrated that preimplantation genetic diagnosis (PGD) can help patients with recurrent miscarriage to reduce their risk of miscarrying (Munné *et al.*, 2005). Also, PGD for translocation carriers was demonstrated to substantially increase a couple's chances of sustaining a pregnancy to full term (Munné *et al.*, 1998, 2000; Verlinsky *et al.,* 2005).

The pregnancy outcome of PGD for translocations has been reported to be lower than regular IVF patients, the reason being the high rate of chromosome abnormalities found in their embryos (Conn *et al.*, 1998; Van Assche *et al.*, 1999; Munné *et al.*, 2000; Gianaroli *et al.*, 2002; Verlinsky *et al.*, 2002). PGD selects against genetic abnormalities that are lethal or that will result in spontaneous abortions, significantly reducing miscarriages in one study from 95% to 13% (Munné *et al.*, 2000), yet there remain few embryos that are normal or balanced, resulting in a low pregnancy rate. Data on 432 cycles of translocation PGD indicates that on average, 72% of embryos from Robertsonian translocation cycles and 82% of reciprocal translocation cycles are abnormal (Munné, unpublished data). Indeed, there is a very good correlation between the percentage of chromosomal abnormalities and pregnancy rate. For instance, cases with >50% abnormal eggs or embryos achieve significantly fewer pregnancies per cycle than cases with <50% abnormal eggs or embryos (Munné *et al.*, 2000). Similarly, Verlinsky *et al.* (2002) found a 28% pregnancy rate in cycles with <75% abnormal embryos compared with 12% in cycles with >75% abnormal embryos.

Cases involving Robertsonian translocations achieve significantly higher pregnancy rates than cases involving reciprocal translocations (Munné *et al.*, 2000; Gianaroli *et al.*, 2003). This is because more abnormal gametes, and therefore abnormal embryos, are produced in reciprocal translocations than in Robertsonian translocations (Munné *et al.*, 2000; Fridstrom *et al.*, 2001; Verlinsky *et al.*, 2002; Gianaroli *et al.*, 2003).

Several articles have evaluated the natural pregnancy outcome of recurrent aborters with translocations (Carp *et al.*, 2004; Goddijn *et al.*, 2004; Sugiura-Ogasawara *et al.*, 2004). In the study of Sugiura-Ogasawara *et al.* (2004), a total of 58 patients were followed after the translocation was diagnosed. The frequency of successful live birth in the first pregnancy was 31.9% (15/47) for reciprocal translocation carriers and 63.6% (7/11) for Robertsonian carriers. According to Sugiura-Ogasawara *et al.* (2004), it takes, on average, 1.3 miscarriages and 23 months for reciprocal translocation carriers to achieve a live-birth pregnancy. However their data does not include in the denominator those couples who stopped trying to conceive because of fear of further miscarriages or became sterile due to repeated miscarriages. Thus, the actual ongoing pregnancy rate for the recurrent miscarriage couples in that study was probably lower than reported. Compared with the outcome of PGD for translocations, the success rate of PGD for Robertsonian translocations (about 50%, Munné *et al.*, 2000; Gianaroli *et al.*, 2003) and for reciprocal translocations (21–23%, Munné *et al.*, 2000; Gianaroli *et al.*, 2003; Verlinsky and Kuliev, 2003) is lower. Even if the results of the study of Sugiura-Ogasawara *et al.* (2004) were to be confirmed in well-controlled studies, that study fails to take into account the pain and suffering of recurrent miscarriage, the fact that missed abortions contribute to sterility in 8% of women (Polishuk *et al.*, 1974), and that intrauterine adhesion, which can cause infertility or further increase in the possibility of miscarriage, is observed in 18.8% of cases who have aborted once and 47.6% who have had recurrent miscarriages (Romer, 1994).

Thus, this study proposed to evaluate the pregnancy outcome of a group of translocation patients who experienced two or more consecutive pregnancy losses and, after several years of trying, had not been able to have a successful pregnancy.

Materials and methods

Subjects

The study sample consisted of couples who were carriers of translocations that had experienced two or more consecutive pregnancy losses and failed to conceive a live birth in their entire reproductive history. Only couples with no previous live births were included in order to be more rigorous about the definition of recurrent miscarriage. All patients were recruited at Otani Women's clinic (Kobe, Japan) between October 2004 and March 2006. Of the 36 potential subjects that underwent PGD cycles, 8.3% (*n* = 3) cancelled the PGD procedure due to poor response to ovarian stimulation, and 33 were included in the study.

Of the 33 who entered into the study, their average maternal age was 32.7 and the mean number of previous aborts was 3.5 ± 1.9.

PGD procedure

After informed and signed consent was obtained from patients, in accordance with Institutional Review Board (IRB) protocol, ovulation stimulation and IVF were performed.

On day 3, each embryo had a single blastomere biopsied, unless the nucleus could not be located after fixation, in which case a second cell was biopsied. Cell fixation was performed as described previously (Velilla *et al.*, 2002). Up to three normally developing embryos that were classified as presumptively chromosomally normal by PGD were transferred on day 4 to 6 or vitrified and transferred in thaw cycle depending on the embryo's and patient's conditions. Abnormal embryos reaching blastocyst stage were frozen on day 5 of development, in case the couple decided later to have these embryos replaced, in accordance to the sensibilities of Japanese society.

Fixed cells were sent to be analysed at Reprogenetics-Japan (Kobe, Japan). Prior to PGD, Reprogenetics-Japan evaluated the blood of the carrier to identify the appropriate DNA probes that would differentiate between balanced or normal cells and unbalanced ones. The strategy for reciprocal translocations, described previously (Munné *et al.*, 2000), consist of using either a combination of two DNA probes that are distal to both chromosome breakpoints, plus one that is proximal, or two proximal and one distal probes. When possible, two distal and two proximal probes were used. Each probe was labelled in a different colour, usually requiring two rounds of fluorescence in-situ hybridization (FISH). Probes were obtained from Abbot (IL, USA).

For Robertsonian translocations, two enumerator probes, one for each chromosome involved in the translocation, were used as described previously (Munné *et al.*, 2000).

Blastomeres showing two signals for each probe were classified as normal or balanced, while any other combination was classified as unbalanced.

Expected versus observed miscarriage rate

In a previous study on recurrent miscarriage (Munné *et al.*, 2005), the pregnancy loss of each subject was compared with that expected based on their own individual history, according to prediction from the study by Brigham *et al.* (1999). However, that study concerns patients without translocations. According to Sugiura-Ogasawara *et al.* (2004), patients who experienced two or more consecutive miscarriages with reciprocal and Robertsonian translocations are expected to lose the next pregnancy at a rate of 68.1% (n = 47) and 36.4% (n = 11), respectively, compared with 28.3% of couples with two or more consecutive miscarriages without translocations. Thus, for purpose of comparison, the results were compared to those of Sugiura-Ogasawara *et al.* (2004).

Implantation was defined as presence of a gestational sac. A spontaneous abortion was defined as loss of a pregnancy after the presence of a gestational sac. An ongoing pregnancy was defined as a pregnancy resulting in a live birth or entered its second trimester, based on the fact that the majority of chromosomally abnormal pregnancies bound to abort are lost in the first trimester (Boue and Boue, 1976; Warburton *et al.*, 1980).

Statistical analysis

Inferences about the observed spontaneous abortion rate after PGD were made by computing the upper confidence limits of the observed rates and comparing the figures with the corresponding statistics observed prior to PGD, for the same group of patients. The computations were based on the binomial distribution. In addition, comparisons of proportions (percentages) were made using Fisher's Exact test.

Results

The 33 couples undergoing PGD had experienced an average of 3.5 previous pregnancies before the PGD cycle, of which 100% (117/117) were lost. Out of these couples, 29 were carriers of reciprocal translocations and 4 had Robertsonian translocations (**Table 1**).

After PGD, 22.7% (20/88) of biopsied embryos were normal or balanced in Robertsonian translocation cases and 17.5% (86/491) in reciprocal translocations. In total, only 18.3% (106/579) embryos were normal or balanced.

After PGD, 5.3% (1/19) of pregnancies were lost. The upper 99% confidence limit on this estimate of the miscarriage rate was 30.2% which is well below the figure of 100% (59/59) observed before PGD for the very same group of patients or the 100% (117/117) for all patients. The results therefore provide convincing evidence (P < 0.001) of a reduced miscarriage rate after PGD. A comparison of the percentages using Fisher's Exact test confirmed this finding (P < 0.001).

According to Sugiura-Ogasawara *et al.* (2004), the expected spontaneous abortion rate in the next pregnancy for 47 couples with reciprocal translocations was 68.1%. After PGD, the rate of spontaneous abortions for reciprocal translocations was 0% (n = 17). The upper 99% point on this estimate was 23.7%, again providing convincing evidence (P < 0.001) of the reduced rate of miscarriage after PGD. The number of cases with Robertsonian translocation was too small to provide meaningful inferences.

Table 2 shows the pregnancy rate obtained after PGD. The pregnancy rate and ongoing pregnancy rate for reciprocal translocations per oocyte retrieval cycle both were 47.2% (17/36), and for Robertsonian translocations, the pregnancy rate and ongoing pregnancy rate were 40% (2/5) and 20% (1/5) respectively.

In total, these 33 patients underwent 1.24 cycles of PGD. The cumulative pregnancy rate was 57.6% and the cumulative ongoing pregnancy rate was 54.5%.

The couples that became pregnant had an average of 3.1 previous miscarriages, compared with 4.3 of those not conceiving, although if one patient with 12 previous miscarriages is not included, the average of previous miscarriages in the group that did not conceive is then 3.6. In addition, the group conceiving had an average maternal age of 31 years compared with 34 for the group not conceiving.

Table 1. Characteristics of patients undergoing preimplantation genetic diagnosis.

Patient number	*No. previous cycles*	*Indication*	*No. previous losses*	*No. previous deliveries*	*Maternal age (years)*
1	2	45,XX,der(13;14)(q10;q10)	3	0	30
2	1	45,XX,der(13;14)(q10;q10)	7	0	41
3	1	45,XX,der(13;14)(q10;q10)	4	0	30
4	1	45,XY,der(14;21)(q10;q10)	3	0	31
5	1	46,XX,(6;17)(p23;q25)	3	0	31
6	2	46,XX,t(1;10)(q43;q22)	3	0	36
7	1	46,XX,t(1;20)(q10;p10)	2	0	29
8	1	46,XX,t(1;7)(q41;q34)	2	0	29
9	1	46,XX,t(10;18)(q11.2;q11.2)	3	0	35
10	1	46,XX,t(12;13)(q15;q14.3)	2	0	34
11	1	46,XX,t(13;21)(q31.2;q21.2)	3	0	35
12	2	46,XX,t(15.22)(q22.3;q13.2)	4	0	29
13	1	46,XX,t(2;3)(p23;p21.3)	2	0	35
14	2	46,XX,t(3;13)(q13.2;q34)	4	0	32
15	1	46,XX,t(6,20)(q22.3;p13)	2	0	32
16	1	46,XX,t(7;16)(q34;q21)	5	0	36
17	1	46,XX,t(7;20)(q21;p11.2)	3	0	35
18	2	46,XX,t(9.10)(q13;q11.2)	6	0	35
19	1	46,XY,t(1;2)(q34.?1;q37.?3)	3	0	33
20	3	46,XY,t(1;21)(p22.1;q22.1)	3	0	26
21	1	46,XY,t(1;6)(q42.1;q22.2)	4	0	27
22	1	46,XY,t(11;16)(q23.1;q22)	2	0	33
23	1	46,XY,t(14;18)t(q32.1;q23)	5	0	29
24	1	46,XY,t(15;17)(q22;p11.2)	2	0	30
25	1	46,XY,t(2;18)(p22;q22)	4	0	33
26	1	46,XY,t(2;7)(p15;q36)	4	0	34
27	1	46,XY,t(5;16)(q22;q22)	4	0	31
28	1	46,XY,t(5;8)(q31.3;p23.1)	2	0	33
29	1	46,XY,t(6;12)(q23.1;p13.2)	3	0	31
30	1	46,XY,t(6;8)(q21;q22)	12	0	34
31	1	46,XY,t(7;11)(q11.2;q25)	2	0	37
32	1	46,XY,t(7;20)(p15;p12)	3	0	36
33	2	46,XY,t(9;17)(q34;q21)	3	0	34
Total			117	0	–
Average			3.5 ± 1.9	0	32.7

Table 2. Results per preimplantation genetic diagnosis cycle.

Translocation type	*Cycles (n)*	*Average age (years)*	*Cycles pregnant (n)*	*Pregnancies (%)*	*Cycles ongoing (n)*	*Cycles lost (n)*	*Lost pregnancies (%)*	*Ongoing pregnancies (%)*
Robertsonian	5	33.0	2	40.0	1	1	50	20.0
Reciprocal	36	32.7	17	47.2	17	0	0	47.2
Total	41	32.7	19	46.3	18	1	5.3	43.9

Discussion

The purpose of this study was to evaluate whether PGD could improve pregnancy outcome in translocation carriers with a history of no previous live births, and two or more consecutive miscarriages. Prevention of all miscarriages was not expected because PGD screens for a limited number of chromosomes, specifically those involved in the translocation, but other chromosome abnormalities not detected could still produce some pregnancy losses.

The key finding was that, for the couples involved in this study, PGD significantly reduced losses and increased the number of viable pregnancies ($P < 0.001$).

The patients had an average of 3.5 previous pregnancies before the PGD cycle, of which 100% were lost. The reported, predicted loss rate in any successive pregnancy for couples who carry reciprocal translocation and who have experienced two or more consecutive miscarriages was 68.1% (Sugiura-Ogasawara *et al*., 2004), although it may be more for patients who never had a previous live birth. Following PGD for reciprocal translocations, the pregnancy loss was 0% ($P < 0.001$). The data confirms previous results, indicating a significant reduction in spontaneous abortions after PGD in recurrent miscarriage patients with and without translocations, as well as in IVF patients without recurrent miscarriage or translocation history (Munné *et al*., 1998, 1999, 2000, 2005, 2006).

The reduction in spontaneous abortions in translocation cases is not surprising if, as in the present study, 18.3% of embryos produced were normal or balanced. Similarly, recurrent miscarriage patients who are non-carriers of translocations have also been reported to have high rates of chromosome abnormalities (Vidal *et al*., 1998; Pellicer *et al*., 1999; Rubio *et al*., 2003) and PGD to significantly reduce miscarriage rates (Munné *et al*., 2005).

If there is little question that PGD can significantly reduce spontaneous abortions, there is some debate regarding the most effective method of producing a live birth, either by natural means disregarding the risk of further miscarriages, or through PGD (Sugiura-Ogasawara and Suzumori, 2005; Munné, 2006). The present study suggests that PGD is superior. First, these patients were not able to produce a live birth before, and after PGD, the ongoing pregnancy rate was 46.2% per cycle or 54.5% per patient, in the short period of time of 1.24 IVF cycles. Second, compared with Sugiura-Ogasawara *et al*. (2004) for reciprocal translocations, the described 32% ongoing pregnancy rate for reciprocal translocation cases compares poorly with the present 47.2% per PGD cycle of reciprocal translocations. This comparison is even better if one takes into account that Sugiura-Ogasawara *et al*. (2004) study did not include in the denominator the patients who did not become pregnant. If, in the present study, only patients who became pregnant were included, the ongoing pregnancy rate would be 100% (17/17).

The effectiveness of PGD for translocations decreased with advancing maternal age, as shown by the fact that the average maternal age of the patients that became pregnant was lower than those that did not. This is because, in addition to the embryos affected by the translocation, there are those embryos affected by aneuploidy that the present test did not screen against.

In conclusion, PGD can benefit translocation carrier couples with a history of not being able to conceive a live child by significantly reducing ($P < 0.001$) spontaneous abortion risk and producing 54.5% ongoing pregnancies in a short period of time.

References

Boue JG, Boue A 1976 Chromosomal abnormalities in early spontaneous abortions (their consequences on early embryogenesis and in vitro growth of embryonic cells). *Current Topics in Pathology* **62**, 193–208.

Brigham SA, Colon C, Farquharson G 1999 A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Human Reproduction* **14**, 2868–2871.

Burgoyne PS, Baker TG 1984 Meiotic pairing and gametogenic failure. In: Evans CW, Dickinson HG (eds) *Controlling events in meiosis*. Proceedings of the 38th Symposium, Reading. Company of Biologists, Cambridge, pp. 349–362.

Carp H, Feldman B, Oelsner G, Schiff E 2004 Parental karyotyping and subsequent live births in recurrent miscarriage. *Fertility and Sterility* **81**, 1296–1301.

Chandley AC 1998 Genetic contribution to male infertility. *Human Reproduction* **13** (Suppl. 3), 76–83.

Chandley AC 1979 The chromosomal basis of human infertility. *British Medical Bulletin* **35**, 181–186.

Conn CM, Harper JC, Winston RML, Delhanty JDA 1998 Infertility couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. *Human Genetics* **102**, 117–123.

Fridstrom M, Ahrlund-Richter, Iwarson E *et al*. 2001 Clinical outcome of treatment cycles using preimplantation genetic diagnosis for structural chromosomal abnormalities. *Prenatal Diagnosis* **21**, 781–987.

Gianaroli L, Magli C, Fiorentino F *et al*. 2003 Clinical value of preimplantiation genetic diagnosis. *Placenta* **24** (Suppl.), S77–S83.

Gianaroli L, Magli MC, Ferraretti AP *et al*. 2002 Possible interchromosomal effect in embryos generated by gametes from translocation carriers. *Human Reproduction* **17**, 3201–3207.

Goddijn M, Joosten JHK, Knegt AC *et al*. 2004 Clinical relevance of diagnosing structural chromosome abnormalities in couples with repeated miscarriage. *Human Reproduction* **19**, 1013–1017.

Hsu LY 1994 Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. *American Journal of Medical Genetics* **53**, 108–140.

Munné S 2006 Preimplantation genetic diagnosis for translocations. *Human Reproduction* **21**, 839–840.

Munné S, Fischer J, Warner A *et al*. and referring centers PGD group 2006 Preimplantation genetic diagnosis significantly reduces pregnancy loss in infertile couples: a multi-center study. *Fertility and Sterility* **85**, 326–332.

Munné S, Chen S, Fischer J *et al*. 2005 Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. *Fertility and Sterility* **84**, 331–335.

Munné S, Sandalinas M, Escudero *T et al*. 2000 Outcome of preimplantation genetic diagnosis of translocations. *Fertility and Sterility* **73**, 1209–1218.

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, Morrison L, Fung J *et al*. 1998 Spontaneous abortions are reduced after pre-conception diagnosis of translocations. *Journal of Assisted Reproduction and Genetics* **15**, 290–296.

Pellicer A, Rubio C, Vidal F *et al*. 1999 In vitro fertilization plus preimplantation genetic diagnosis in patients with recurrent miscarriage: an analysis of chromosome abnormalities in human preimplantation embryos. *Fertility and Sterility* **71**, 1033–1039.

Polishuk WZ, Schenker JG, Yarkoni S 1974 Menstrual and obstetric sequelae of missed abortion. *Acta Europaea Fertilitatis* **5**, 289–293.

Romer T 1994 Post-abortion-hysteroscopy – a method for early diagnosis of congenital and acquired intrauterine causes of abortions. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **57**, 171–173.

Rubio C, Simón C, Vidal F *et al*. 2003 Chromosomal abnormalities and embryo development in recurrent miscarriage couples. *Human Reproduction* **18**, 182–188.

Sugiura-Ogasawara M, Suzumori K 2005 Can preimplantation genetic diagnosis improve success rates in recurrent aborters with translocations? *Human Reproduction* **20**, 1–4.

Sugiura-Ogasawara M, Ozaki Y, Sato *T et al*. 2004 Poor prognosis of recurrent aborters with either maternal or paternal reciprocal translocations. *Fertility and Sterility* **81**, 367–373.

Tupler R, Barbierato L, Larizza D *et al*. 1994 Balanced autosomal translocations and ovarian dysgenesis. *Human Genetics* **94**, 171–176.

Van Assche E, Staessen C, Vegetti W *et al*. 1999 Preimplantation genetic diagnosis and sperm analysis by fluorescence in-situ hybridization for the most common reciprocal translocation t(11;22). *Molecular Human Reproduction* **5**, 682–690.

Velilla E, Escudero T, Munné S 2002 Blastomere fixation techniques and risk of misdiagnosis for PGD of aneuploidy. *Reproductive BioMedicine Online* **4**, 210–217.

Verlinsky Y, Kuliev A 2003 Thirteen years' experience of preimplantation diagnosis: report of the fifth international symposium on preimplantation genetics. *Reproductive BioMedicine Online* **8**, 229–235.

Verlinsky Y, Tur-Kaspa I, Cieslak J *et al*. 2005 Preimplantation testing for chromosomal disorders improves reproductive outcome of poor-prognosis patients. *Reproductive BioMedicine Online* **11**, 219–225.

Verlinsky Y, Cieslak V, Evsikov G *et al*. 2002 nuclear transfer for full karyotyping and preimplantation diagnosis for translocations. *Reproductive BioMedicine Online* **5**, 300–305.

Vidal F, Gimenez C, Rubio C *et al*. 1998 FISH preimplantation diagnosis of chromosome aneuploidy in recurrent pregnancy wastage. *Journal of Assisted Reproduction and Genetics* **15**, 310–313.

Warburton D, Stein Z, Kline J, Susser M 1980 Chromosome abnormalities in spontaneous abortion: data from the New York city study. In: Porter LH, Hook EB (eds) *Human embryonic and fetal death*. Academic press, New York, pp. 261–287.

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