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Poor embryo development and preimplantation genetic diagnosis outcomes of translocations involving chromosome 10: Do we blame genetics?

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Summary

Balanced reciprocal translocation carriers are usually phenotypically normal. Although the reproductive risk of these carriers varies, they generally have a lower chance to produce normal or balanced gametes. Preimplantation genetic diagnosis (PGD) is offered to these patients to increase their chances of becoming pregnant by selecting a balanced embryo for transfer. This study aimed to analyse the development and the PGD outcome of the embryos obtained from reciprocal translocation carriers focusing on ones with chromosome 10 rearrangements. In total, 27 reciprocal translocation carriers underwent 31 cycles of PGD. PGD was performed using multicolour fluorescence *in situ* hybridisation for 298 embryos and of these 136 were obtained from couples carrying translocations involving chromosome 10 rearrangements. Carriers of translocations involving chromosome 10 rearrangements have a lower chance of producing normal or balanced embryos compared with the carriers with other rearrangements. The development of embryos obtained from the patients with chromosome 10 rearrangements was impaired and only a limited number of embryos developed to the blastocyst stage.

Keywords: Chromosome 10, Embryo development, Human embryo, PGD, Reciprocal translocation

Introduction

Balanced reciprocal translocations are the most common chromosome rearrangements occurring 1 in 500 live births (Jacobs *et al.*, 1974). Carriers of reciprocal translocations are usually phenotypically normal; however they can have a lower chance to produce normal or balanced gametes leading to repeated spontaneous abortions and infertility (Scriven *et al.*, 1998; Simopoulou *et al.*, 2003). The chances of these carriers developing a balanced embryo depends on the positions of the breakpoints, the segregation of the rearranged chromosomes, type of translocation and gender of the carrier (Lim *et al.*, 2008; Lledo *et al.*, 2010). The chance of male carriers to produce a balanced sperm was shown to vary between 20 and 80% (Escudero *et al.*, 2003; Munne, 2005). Analysis of the segregation modes in females was proven to be more difficult and it has been restricted to fetal ovarian tissue (Hartshorne *et al.*, 1999) and embryos derived from female carriers undergoing preimplantation genetic diagnosis (PGD) (Munne *et al.*, 2000; Ko *et al.*, 2013). It is important to understand the meiotic segregation in embryos derived from translocation carriers and examine the possible effect of this segregation in preimplantation embryo development to estimate the risk of implantation failure and pregnancy loss. Preliminary studies in our centre showed that the chance of an embryo transfer for a reciprocal translocation carrier is lower compared with the other patients with no chromosomal rearrangements. These carriers were also shown to have lower rates of pregnancies. It was observed that these low pregnancy rates are mainly originated from carriers with chromosome 10 rearrangements. Therefore we sought to investigate the segregation patterns in embryos derived from reciprocal translocation carriers

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focusing on rearrangements involving chromosome 10. We further investigated the development of embryos derived from these patients.

Materials and methods

Patient information

In total, 27 couples underwent 31 cycles of PGD for reciprocal translocations from August 2010 to May 2013 in Bahceci Assisted Reproductive Technology Centre. All the patients' karyotypes were defined by clinical cytogeneticists using standard chromosome banding techniques. Informed consent was obtained from all the couples prior to each PGD cycle and institutional review board (IRB) approval was obtained from Bahceci Assisted Reproductive Technology Centre. Work-up was performed using the patients' lymphocytes before the stimulation process started. Fluorescence *in situ* hybridisation (FISH) was optimised using one centromeric and two subtelomeric probes targeting the translocated segments of the chromosomes. Karyotypes of each patient with probes used for each PGD case are summarised in Table 1.

Ovarian stimulation, embryo culture and blastomere biopsy

Controlled ovarian stimulation was performed as described previously (Ulug *et al.*, 2007). Briefly, ovulation was induced using human chorionic gonadotrophin (hCG) injection (Ovidrelle; Merck Serono, UK). Follicles were aspirated 35–36 h post hCG injection under ultrasound guidance and oocytes were retrieved. After at least 2 h of oocyte culture, hyaluronidase treatment was performed. Discontinuous colloidal silica gel gradient (PureSperm; Nidacon, Sweden) was used to process the semen samples and the sperm pellet was washed twice with sperm washing medium. Only meiosis II (MII) stage oocytes underwent the intracytoplasmic sperm injection (ICSI) procedure. ICSI procedure was performed in mHTF solution containing HEPES. Injected oocytes were cultured in Single Step Media (SSM) supplemented with 10% synthetic serum (Irvine Scientific, Irvine, CA, USA) in a 5% CO_2 and 5% O_2 in air incubator (INB-203C, IKS International, The Netherlands). Fertilisation checks were performed 14–16 h post ICSI. Failed and abnormal fertilisation, which was defined as one pronucleus (1PN) or more than two pronuclei (≥3PN), was recorded. Embryo morphology was examined on day 3 of development and the number of cells, presence of even and uneven cells and expansion of cells were recorded. Only good quality embryos with at least six cells and less than 50% fragmentation were biopsied for PGD. Single blastomere was biopsied from these embryos on day 3 of development by breaching of the zona pellucida with a laser (Octax^{TM}, MTG, Germany). Culture medium was replenished on day 3 after the biopsy and embryos were kept in these conditions until the day of embryo transfer. The development of embryos obtained from age-matched patients with no chromosomal rearrangements was analysed as a control group.

Blastomere spreading and fluorescence *in situ* hybridisation

In total, 298 nuclei from single blastomeres were fixed on poly-L-lysine-coated slides (Thermo Scientific, Germany). A combination of alpha satellite and subtelomeric probes to translocated segments was used to analyse the chromosomes involved in the translocation by FISH (Table 1). The nuclei were visualised under an Olympus fluorescence microscope. All the nuclei were evaluated by two experts and the type of meiotic segregation of embryos was determined. The diploid blastocysts were transferred into the uterus of the patient on day 5 of embryo development.

Statistical analysis

Statistical analyses were performed by chi-squared test using GraphPad Prism v6 software. Chi-squared test was performed to determine if there were significant differences in maturation of oocytes, normal fertilisation rates and progression of development in embryos obtained from carriers of reciprocal translocations.

Results

In total, 27 patients underwent 31 cycles of PGD for reciprocal translocations. The maturation of oocytes and development of embryos obtained from these carriers were analysed. In the same time period, the oocyte maturation and development of embryos obtained from 60 age-matched patients with no chromosomal rearrangements were analysed as a control group. Overall, 733 oocytes were collected from reciprocal translocation carriers and 84% (617) of these oocytes were microinjected by the ICSI procedure. Of these injected oocytes, 78% (482) were fertilised normally (Table 2). In the control group, 594 oocytes were collected and 93% (551) of the oocytes were microinjected. Of these fertilised oocytes 79% (438) were fertilised normally.

Overall, there were no differences in the maturation and fertilisation of the oocytes obtained from the reciprocal translocation carriers compared with the

Table 1 List of patient information. Patient ID with the karyotype, maternal age at the time of oocytes retrieval and the list of probes used in PGD analyses are listed

Patient ID	Karyotype	Indication	Maternal age	FISH probes used
1	46,XY,t(1;10)(p22;q22)	Translocation, Previous IVF failure	38	LPT 1p (Red), CEP 10 (Spectrum Aqua), LPT 10q (Green)
2	46,XX,t(1;10)(p32;q21.3)	Translocation, Previous IVF failure	40	LPT 1p (Red), CEP 10 (Spectrum Aqua), LPT 10q (Green)
3	46,XX,t(1;10)(p?34;p11.2)	Translocation	34	LPT 1p (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
4	46,XY,t(2;10)(q?23;p?13)	Translocation	31	LPT 2q (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
5	46,XY,t(3;10)(p21.3;p15)	Translocation, Previous IVF failure	33	LPT 3p (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
6	46,XX,t(4;10)(q31.2;q21.2)	Translocation, Previous IVF failure	32	TelVysion 4q (Spectrum Orange), CEP 10 (Spectrum Aqua), LPT 10q (Green)
7	46,XY,t(5;10)(q13;q24)	Translocation	33	LPT 5q (Red), CEP 10 (Spectrum Aqua), LPT 10q (Green)
8	46,XX,t(7;10)(p13;p11.2)	Translocation, Previous IVF failure	25	LPT 7p (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
9	46,XY,t(9;10)(p13;q11.2)	Translocation, Previous IVF failure	34	LPT 9p (Red), CEP 9 (Spectrum Aqua), LPT 10q (Green)
10	46,XX,t(10;12)(q26;q24)	Translocation, Previous IVF failure	28	LPT 10q (Green), CEP 10 (Spectrum Aqua), TelVysion 12q (Spectrum Orange)
11	46,XX,t(10;14)(q22.3;q13)	Translocation, Previous IVF failure	27	LPT 10q (Green), CEP 10 (Spectrum Aqua), LPT 14q (Red)
12	46,XY,t(1;2)(p?36;p?14–16)	Translocation	39	LPT 1p (Green), CEP 1 (Spectrum Orange), LPT 2p (Red)
13	46,XX,t(1;5)(q22;q23)	Translocation	30	LPT 1q (Green), CEP 1 (Spectrum Orange), LPT 5q (Red)
14	46,XY,t(1;6)(q23.1;q21)	Translocation, Previous IVF failure	30	LPT 1q (Green), CEP 1 (Spectrum Orange), LPT 6q (Red)
15	46,XY,t(1;16)(q21;q12)	Translocation, Previous IVF failure	41	LPT 1q (Red), CEP 1 (Spectrum Orange), LPT 16q (Green)
16	46,XY,t(1;22)(q12;q?11.2)	Translocation, Previous IVF failure	39	LPT 1q (Red), CEP 1 (Spectrum Orange), LPT 22q (Green)
17*	46,XY,t(2;5)(p11.2;q33)	Translocation, Previous IVF failure	36	LPT 2p (Red), LPT 2q (Green), LPT 5q (Red)*
18*	46,XY,t(3;5)(q12;p12)	Translocation	30	LPT 3p (Green), LPT 3q (Red), LPT 5p (Red)*
19	46,XX,t(5;20)(q31;q13.3)	Translocation, Previous IVF failure	37	LPT 5q (Red), TelVysion 20p (Spectrum Green), TelVysion 20q (Spectrum Orange)
20*	46,XY,t(5;22)(q22;qter)	Translocation, Previous IVF failure	33	LPT 5p (Red), LPT 5q (Red), LPT 22q (Green), LSI 22(Spectrum Green)*
21	46,XY,t(8;11)(q21.2;q23)	Translocation	38	LPT 8q (Red), CEP 8 (Spectrum Aqua), TelVysion 11q (Spectrum Orange)
22*	46,XY,t(8;22)(q23.2;qter)	Translocation, Previous IVF failure	28	LPT 8q (Red), CEP 8 (Spectrum Aqua), LPT 22q (Green), LSI 22 (Spectrum Green)*
23	46,XY,t(9;12)(q22;q24.3)	Translocation, Previous IVF failure	36	TelVysion 9q (Spectrum Orange), CEP 9 (Spectrum Aqua), LPT 12q (Green)
24	46,XX,t(9;20)(q34;q13)	Translocation	30	TelVysion 9q (Spectrum Orange), CEP 9 (Spectrum Aqua), LSI 20q (Spectrum Gold)
25	46,XX,t(11;22)(q11.2;q13.3)	Translocation	32	TelVysion 11q (Spectrum Orange), CEP 11 (Spectrum Aqua), LPT 22q (Green), LSI 22 (Spectrum Green)*
26*	46,XY,t(13;22)(p11.2;q13.1)	Translocation, Previous IVF failure	26	LPT 13q (Red), LPT 22q (Green), LSI 13 (Spectrum Orange), LSI 22 (Spectrum Green)*
27*	46,XY,t(16;22)(q22;q13)	Translocation, Previous IVF failure	36	LPT 16q (Green), CEP 16 (Spectrum Aqua), LPT 22q (Green)*

All the probes are from Cytocell (UK) and Abbott Molecular Inc. (USA).
*Represents two rounds of FISH analysis. IVF, *in vitro* fertilisation.

Table 2 Summary table for patient background. The maternal and paternal age, the number of previous IVF treatments and the number of previous miscarriages are listed

				Translocation carriers with chromosome 10 rearrangements		
	Control group (patients with no chromosomal rearrangements)	Total reciprocal translocation carriers (number of embryos)	Translocation carriers with no chromosome 10 rearrangements	Chromosome 10 patients	Male carriers	Female carriers
Patients	60	27	16	11	5	6
Cycles	60	31	16	15	6	9
Average maternal age	41	33.5	34	33	34	32
Average paternal age	43	36.5	38	35	34	36
Previous IVF cycles	0.2	2	0.4	2.1 (0–6)	1.5 (0–3)	2.5 (0–6)
Previous miscarriages	0.5	0.6	0.4	0.8 (0–2)	0.5 (0–1)	1 (0–2)

IVF, *in vitro* fertilisation.

control group. Once we analysed the progression of embryo development, it was observed that the embryos derived from carriers of translocations with chromosome 10 rearrangements was developing slower, in such only 24% (26/109) of the embryos obtained from these carriers reached the blastocyst stage (Table 3). Moreover, distinct differences were observed in the progression of embryo development between the male and female translocation carriers with chromosome 10 rearrangements. None of the embryos obtained from the male carriers with chromosome 10 rearrangements developed to the blastocyst stage, whereas 28% (26/94) of embryos derived from female carriers reached the blastocyst stage ($P < 0.0001$). We further investigated the meiotic segregation of the chromosomes in these embryos obtained from reciprocal translocation carriers with chromosome 10 rearrangements. It was observed that only 12% (16/135) of the embryos were balanced (Table 4) and despite the developmental incompetence in the embryos obtained from male carriers, the prevalence of 2:2 alternate segregation mode was slightly higher in these embryos compared with the ones obtained from female carriers (8 versus 5%, respectively, $P = 0.5$). The most common segregation pattern in the embryos obtained from these carriers with chromosome 10 rearrangements was shown to be the unbalanced 3:1 mode (44%, 59/135).

Discussion

In this study, we investigated the progression of embryo development and PGD outcome in embryos obtained from reciprocal translocation carriers. A high number of patients was admitted to our clinic due to translocations involving chromosome 10 and thus our analysis was focused on the embryos obtained from these carriers with chromosome 10 rearrangements. We observed that the maturation of oocytes was similar among the control group and reciprocal translocation carriers. However, embryo development was significantly compromised in the male carriers with chromosome 10 rearrangements. The female partners of these carriers did not have an infertility problem; the detailed indication for each patient is listed in Table 1. Previous studies analysing the progression of embryo development derived from reciprocal translocation carriers showed that embryonic development may be impaired (Findikli *et al.*, 2003). However contradictory studies focusing on only the unbalanced embryos showed that development was not disturbed and that these embryos were capable of reaching the blastocyst stage (Evsikov *et al.*, 2000). It is well known that the embryo undergoes gradual parental demethylation

Table 3 Summary table showing the number of oocytes retrieved with the number of meiosis II (MII) stage oocytes, fertilised oocytes (2PN) and biopsied embryos are listed

		Total reciprocal translocation carriers (number of embryos)	Translocation carriers with chromosome 10 rearrangements			Translocation carriers with no chromosome 10 rearrangements		
	Control groups ICSI patients	Total	Total	Female	Male	Total	Female	Male
Total oocytes	594	733	339	224	115	394	74	320
MII	551	84% (617)	85% (288)	81% (191)	84% (97)	84% (329)	88% (65)	83% (264)
2PN	438	78% (482)	84% (241)	83% (158)	72% (83)	73% (241)	75% (49)	73% (192)
Blastocyst development	22% (96/438)	28% (52/189)	24% (26/109)	28% (26/94)	0% (0/15)	33% (26/80)	31% (5/16)	33% (21/64)

Table 4 Summary of PGD cycle. Number of biopsied, diagnosed and transferable embryos are listed. Number of patients with positive β-hCG are also listed

	Control groups ICSI patients	Total reciprocal translocation carriers (number of embryos)	Translocation carriers with chromosome 10 rearrangements			Translocation carriers with no chromosome 10 rearrangements
			Chromosome 10 patients	Female carriers	Male carriers	
Biopsied embryos	N/A	62% (298/482)	57% (136/241)	54% (86/158)	60% (50/83)	67% (162/241)
Diagnosed embryos for translocation	N/A	99% (294/298)	99% (135/136)	99% (85/86)	100% (50/50)	98% (159/162)
Transferable embryos	45% (43/96)	15% (43/294)	6% (8/135)	5% (4/85)	8% (4/50)	22% (35/159)
Positive β-hCG per cycle	36% (21/57)	13% (4/31)	7% (1/15)	11% (1/9)	0% (0/6)	19% (3/16)

N/A, not applicable.

at the early cleavage divisions (Mayer *et al.*, 2000; Oswald *et al.*, 2000; Reik *et al.*, 2001; Beaujean *et al.*, 2004) with the rapid demethylation of the paternal genome (Rougier *et al.*, 1998; Mayer *et al.*, 2000; Oswald *et al.*, 2000; Dean *et al.*, 2001; Santos *et al.*, 2002; Beaujean *et al.*, 2004; Santos & Dean, 2004) and gradual demethylation of the maternal genome (Monk *et al.*, 1987; Howlett & Reik, 1991; Rougier *et al.*, 1998; Santos *et al.*, 2002; Beaujean *et al.*, 2004; Santos & Dean, 2004). Therefore, it is possible that the development of an embryo with an unbalanced chromosome complement originated paternally is more compromised due to the rapid demethylation process. It is also possible that in our study the developmental incompetence of the embryos derived from male carriers with chromosome 10 rearrangements is more exaggerated due to the small number of embryos analysed.

The PGD outcome also showed that the likelihood of obtaining a balanced embryo from carriers with chromosome 10 rearrangements was low. The most common segregation pattern observed in these embryos was the unbalanced 3:1 segregation with an increased rate in embryos obtained from male carriers than female carriers. Diploid sperm could be one of the causes of this result as Van Hummelen and colleagues (1997) showed a higher frequency of diploid sperm in a translocation carrier involving chromosomes 1 and 10 rearrangement (Van Hummelen *et al.*, 1997). This increased rate was suggested to be due to abnormal chromosome pairing and the frequency of chiasmata leading to the absence of cytokinesis and resulting in diploid sperm (Goldman *et al.*, 1993; Kleckner, 1996). Therefore, further analysis of sperm FISH in male carriers with chromosome 10 rearrangements may shed light into the underlying reasons of obtaining such a high number of unbalanced embryos.

The main limitation of this study was the small number of embryos analysed and the technique used in PGD. Although recently comprehensive chromosomal screening using aCGH has emerged, FISH is still used to perform PGD for translocations (Scriven *et al.*, 2013; Van Echten-Arends *et al.*, 2013). One of the reasons for this is that, depending on the breakpoints involved in the translocation, the detection sensitivity by aCGH varies. In this study, only one of the translocated segments could be detected in 33%; in 11% of the carriers none of the translocated segments could be detected even with the high resolution aCGH platforms (24 Sure+). Although high resolution aCGH could have been used in the diagnosis of the embryos where one translocated segment could be detected, there still was a risk of misdiagnosis in cases due to possible hybridisation failures of the probes on the microarray. Therefore, in our centre, we only perform PGD by aCGH when both of the translocated segments could be identified. In addition to the limitations of aCGH detection sensitivity, in most of the newly developing countries FISH is preferred for PGD for translocations due to the high costs of aCGH.

In conclusion these data show that a carrier of a translocation with chromosome 10 rearrangement has a lower chance of producing a normal or balanced embryo and that the gender of the carrier might also be an indicator of whether there is a higher chance of finding a normal or balanced embryo for transfer.

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Conflict of interest statement

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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