SUPERIOR A.R.T.
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Pre-implantation genetic screening for discrimination of carrier and non-carrier reciprocal translocation with array-CGH and PGD

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Introduction

A reciprocal translocation is an unusual arrangement of chromosome, typically an exchange of two chromosomal segments from two non-homologous chromosomes, leading to less embryos of normal euploid status, increased failed implantations and recurrent miscarriages, and some liveborn with serious health problems. Pre-implantation genetic screening (PGS) with array-Comparative Genomic Hybridization (CGH) is currently in clinical use for detecting reciprocal translocations. However, CGH cannot differentiate between euploid embryos carrying and not carrying the balanced. Pre-implantation genetic diagnosis (PGD) using molecular techniques was performed post-CGH on whole genomic amplification (WGA) material to further distinguish euploid embryos as carriers or non-carriers of the balanced reciprocal translocation.

Methods & Materials

Review & Samples Preparation; Karyotypes were requested for each couple. Prior to performing translocation analysis with 24sure+ slides, the position of translocation chromosome was used to calculate probability of detection using 24sure+TL program (Illumina). Blood samples of couples and their child were collected with informed consent. DNA extraction was performed using The Wizard® Genomic DNA Purification Kit (Promega).

Array CGH; Following an IVF cycle, a single biopsy of day 5 or 6 trophectoderm were amplified to whole genomic amplification (WGA) using SurePlex DNA amplification system, was processed using 24 Sure^{+} slides according to manufacturer's instructions (Illumina) and was analyzed with Bluefused multisoftware v. 4.3.

Post-CGH analysis; The precise breakpoints of translocation were derived from embryo carrying unbalanced translocation resulted from array CGH. Short tandem repeat (STR) marker was designed close to and straddling the breakpoints of chromosomal translocation.
Balanced and unbalanced translocation embryo(s), including couples and their child samples were amplified by set of selected informative STR polymorphic marker. Genetic material from these embryos were analyzed together to see and discriminate which, if any, of carrier and non-carrier balanced translocation embryo.

Results

Two couples carrying different balanced translocations; 46XX,t(7;8)(q11.23;q21.2) in first couple and 46XY,t(10;12)(q26;p11.2) in second couple were reviewed in this study. Prior to performing translocation analysis with 24sure+ slides, the position of translocation chromosome was used to calculate probability of detection with 24sure+ slide. The overall probability of detection is 0.9996 and 0.98 for first couple and second couple, respectively.
PGS-CGH/TL was performed in 12 embryos and the result shown 8 embryos (67%) revealing unbalanced and abnormal pattern. (Table 1).

Table 1. PGS-CGH and PGD-PCR results of embryo from two couples carrying reciprocal translocation.

Case-cycle	Karyotype of translocation carrier	Total no. of embryo	Balanced translocation		Unbalanced Translocation	Aneuploidy	ET	Child born
			Carrier	Non -carrier				
C1-1	46XX;t(7;8)(q11.23;q21.2)	6	1*	1	2	2	1	1
C2-1	46XY;t(10;12)(q26;p11.2)	6	1	1*	1	3	1	0

* this embryo was transferred.

The precise breakpoints of translocation were derived from embryo carrying unbalanced translocation (Figure 1 and 2). The STR markers in flanking of breakpoint were designed and used in post-CGH analysis (Table 2).

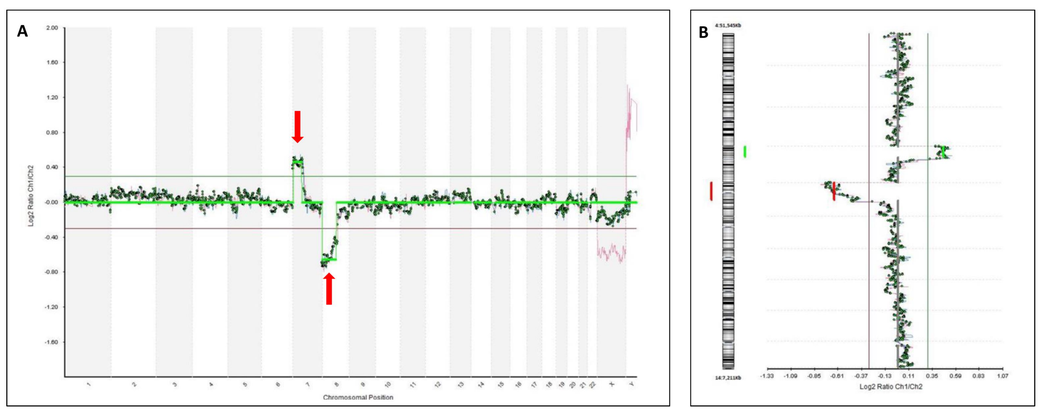

Figure 1. CGH analysis of an unbalanced sample with 46XX;t(7;8)(q11.23;q21.2) in first couples (1A and 1B).

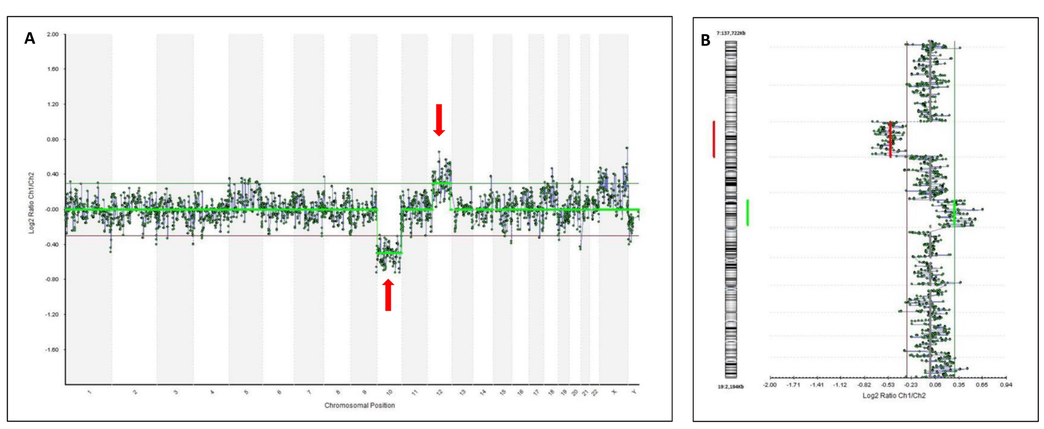

Figure 2. CGH analysis of an unbalanced sample with 46XY;t(10;12)(q26;p11.2) in second couples (2A and 2B).

Table 2. STR markers in flanking of breakpoint for post-CGH analysis.

Case	Chromosome	Markers
1	7q11.23	D7S2516, D7S653, D7S672, D7S2518, D7S2470,D7S2455
	8q21.2	D8S275, D8S525, D8S1697, D8S167, D8S271, D8S273
2	12p11.2	D12S1617,D12S1034, D12S1640, D12S1631,D12S87, D12S1648

Post-CGH analysis was processed on the WGA of 4 balanced embryo (2 embryo/family). The results shown 1 carrier and 1 non-carrier from each family. The carrier embryo was transferred in the first family leading to the birth of a healthy carrier female. The second family transferred a non-carrier embryo, but pregnancy was not achieved. The second family consequently underwent a further two IVF/PGS-CGH/TL treatment cycles with 6 embryos. All were unbalanced for the reciprocal translocation and therefore not suitable for transfer.

Discussions & Conclusion

- The accuracy of karyotype is very important for PGS-CGH/TL because the position of translocation chromosome was prior to calculate probability of detection with 24sure+ slide. The probability of detecting an unbalanced translocation, and therefore the success of the array-CGH-based analysis, is dependent upon the location of the translocation breakpoints in the chromosomes and the size of the unbalanced region (1). Then, the STR markers were designed flanking and straddling of breakpoint.
- The obstacle of post-CGH analysis is allele drop out (ADO) found in some markers when amplified with WGA. However, the use of a strategy involving at least three markers besides on breakpoint reduces this risk substantially (2).
- The limitation of this technique is the breakpoint of translocation chromosome could not derive when it does not have unbalance translocation embryo in that IVF cycles.
- The results indicated that the option of PGS-CGH/TL using 24 sure^{+} can accurately evaluate the breakpoint to allow further diagnosis by PGD-PCR. It is a feasible approach to obtain embryos with a normal chromosome complement and increased the chance for a successful live birth. However, post-CGH PGD-PCR analysis still has limitations in some families.

References

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