The First Live Birth in Hong Kong Following Preimplantation Genetic Diagnosis for Robertsonian Translocation Using Array Comparative Genomic Hybridisation

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The use of preimplantation genetic diagnosis has been available in Hong Kong for more than 10 years. In the past, fluorescence in-situ hybridisation technique was used for preimplantation genetic diagnosis for translocation carriers. Array comparative genomic hybridisation was developed with the advantages of testing all 24 chromosomes and being a user-friendly technique. We report the first live birth in Hong Kong after preimplantation genetic diagnosis for Robertsonian translocation using array comparative genomic hybridisation.
Hong Kong J Gynaecol Obstet Midwifery 2015; 15(1):93-6

Keywords: Comparative genomic hybridization; Preimplantation diagnosis; Translocation, genetic

Introduction

We would like to report the first live birth in Hong Kong following preimplantation genetic diagnosis (PGD) for Robertsonian translocation using array comparative genomic hybridisation (aCGH).

Case Report

A 34-year-old patient was referred to our subfertility clinic in 2009 for primary severe male factor subfertility. Repeated semen analysis of her husband revealed severe oligozoospermia, with sperm concentration of <1 million/ml. Thus, the couple was advised to undergo in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) treatment. Karyotyping of the husband showed 45,XY,rob(14;15)(q10;q10) and no Y chromosome microdeletion. In view of the balanced translocation, PGD using fluorescence in-situ hybridisation (FISH) was offered after extensive counselling.

The first IVF/PGD cycle was performed in July 2010. After 9 days of ovarian stimulation, eight oocytes were retrieved and four were fertilised after ICSI of seven mature oocytes. Embryo biopsy done on four day-3 embryos showed one embryo with normal FISH signals. On day 5, the embryo was of fair quality at morula stage and was transferred. However, the patient failed to conceive.

The second IVF/PGD cycle was performed in December 2010. After 9 days of ovarian stimulation, 18 oocytes were retrieved and 13 were fertilised after ICSI of 17 mature oocytes. Embryo biopsy of 10 day-3 embryos showed four embryos with normal FISH signals. One morula and one early blastocyst of fair quality were transferred on day 5. The patient became pregnant but this ended up in a biochemical pregnancy.

The third IVF/PGD cycle was performed in July 2011. After 8 days of ovarian stimulation, 18 oocytes were retrieved and 16 were fertilised after ICSI for 16 oocytes. Eleven day-3 embryos were available for embryo biopsy. It showed three embryos with normal FISH signals. Two embryos were of good quality on day 5 and were transferred. The patient became pregnant but the pregnancy ended again as an early spontaneous miscarriage.

After further extensive counselling, she decided to

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proceed to the fourth IVF/PGD cycle in May 2012. After 8 days of ovarian stimulation, 25 oocytes were retrieved and 16 were fertilised after ICSI of 24 oocytes. Embryo biopsy was done on 13 day-3 embryos. In April 2012, we acquired the platform of aCGH for translocation. As only nine sets of FISH probes were available, the couple was counselled to use aCGH for the remaining four embryos. We found three embryos with normal FISH signals (Figure 1) and three embryos with no aneuploidy on aCGH (Figure 2). One morula and one blastocyst of grade 4BB, both after aCGH, were transferred on day 5 and the patient became pregnant with a singleton pregnancy. One blastocyst of grade 5BB, after aCGH, was vitrified on day 6. She declined invasive prenatal diagnosis testing because of the associated risk of miscarriage. She delivered a healthy and phenotypically normal baby boy in February 2013. Cord blood analysis revealed the karyotype of the baby boy to be 46,XY, which was compatible with our PGD results; no uniparental disomy was detected.

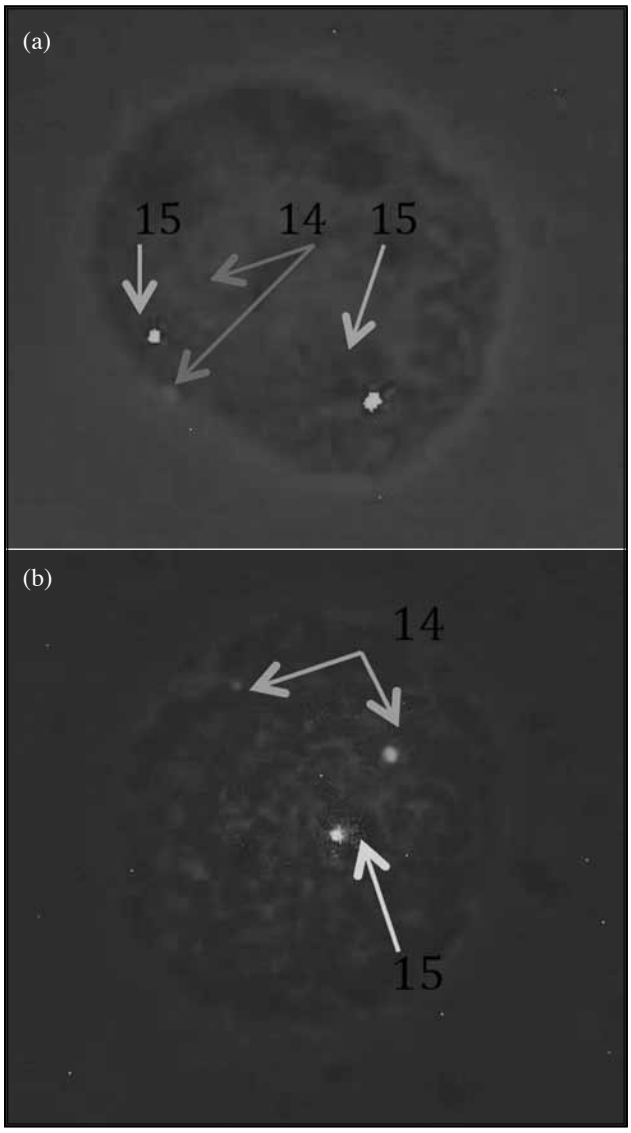

Figure 1. An embryo with (a) normal and (b) abnormal fluorescence in-situ hybridisation signals

Embryo Biopsy and Preimplantation Genetic Diagnosis Treatment Cycles

One blastomere was biopsied from each good-quality day-3 embryo. The blastomere was either fixed for FISH analysis or underwent aCGH according to the manufacturer's protocol (BlueGnome, UK). For FISH analysis, Cytocell (UK) 14qter (red) and Cytocell 15qter (green) probes were used. Two laboratory staffs independently interpreted the FISH results.

Discussion

The use of PGD for sex-linked disease in the first-born baby in the world was reported in 1990[1], followed by another baby born after PGD of cystic fibrosis 2 years later[2]. Embryo biopsy is usually performed on day 3 after oocyte retrieval and one or two blastomere biopsies were used for the genetic testing. Subsequently, there was increasing use of the technique for both monogenetic diseases using polymerase chain reaction (PCR) and translocation carriers using FISH. Aneuploidy screening (preimplantation genetic screening [PGS]) for embryos before transferring back to the uterus in some at-risk groups of women, such as those with advanced maternal age and recurrent pregnancy loss, was advocated to increase the pregnancy rate and reduce miscarriage rate. In our unit, we have been offering PGD treatment for more than 10 years and reported the first case of PGD using FISH[3]. We use blastomere biopsy together with PCR and FISH for monogenetic diseases and translocation carriers, respectively. An increasing number of PGD cycles have been performed in this decade.

Fluorescence in-situ hybridisation has been used for translocation and aneuploidy screening but it can only test for five to nine chromosomes, at most 15 chromosomes, in repeated rounds, as there is a limited number of spectrally distinct fluorochromes (colours) available for labelling of DNA probes. However, the accuracy of FISH analysis decreases with each additional round of hybridisation[4]. Moreover, as shown by evidence using an array-based approach on the remaining blastomeres from embryos after PGD, aneuploidies and chromosomal rearrangements, including chromosome breakage leading to segmental aberrations, were not picked up by the traditional FISH technique[5-7]. Moreover, there is technical difficulty with FISH technique itself[4] and an error rate of 7% to 10% has been estimated[8,9]. In translocation carriers, there is evidence that interchromosomal effect may increase

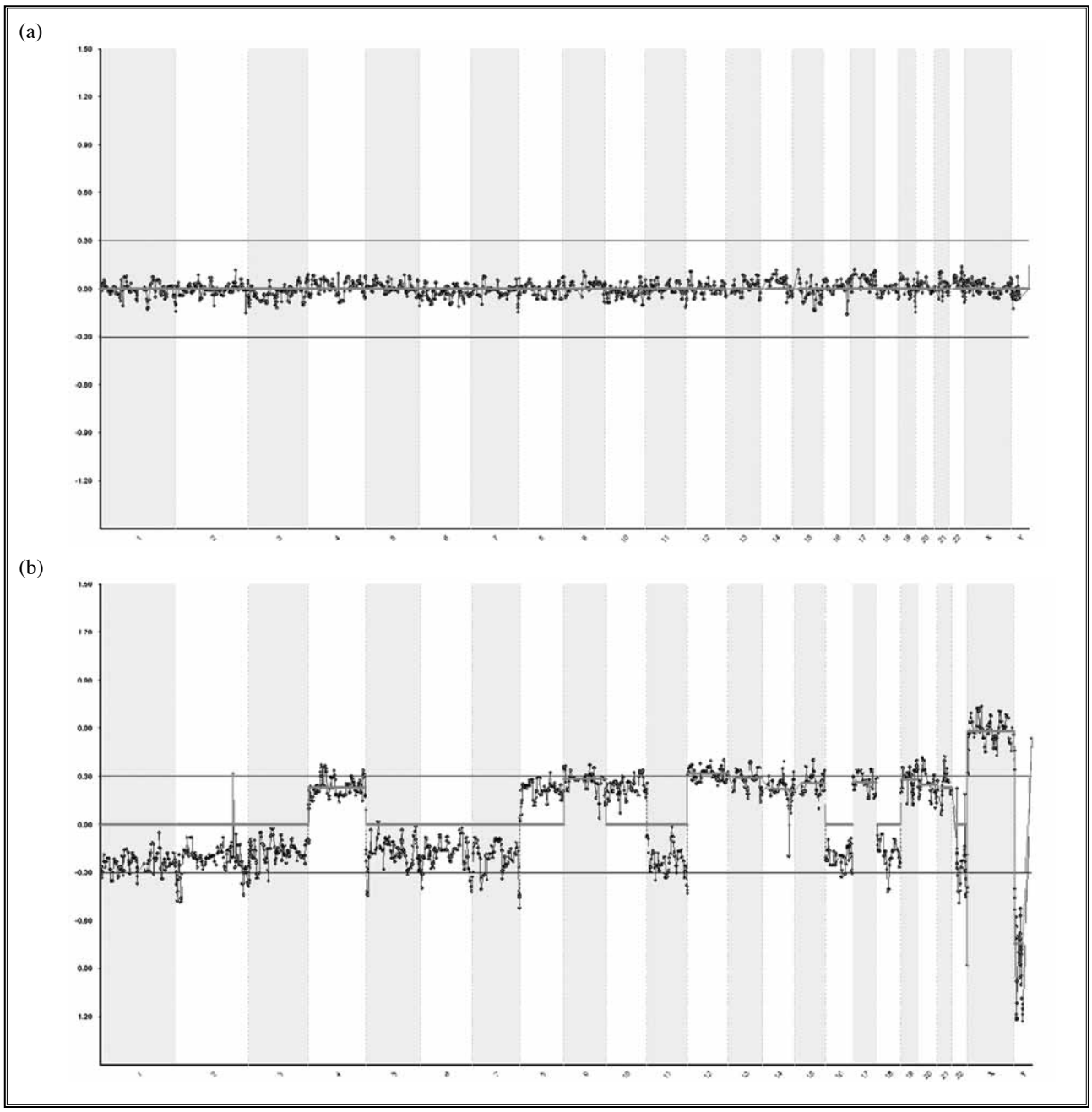

Figure 2. (a) Array comparative genomic hybridisation (aCGH) result of an embryo which resulted in a singleton live birth after transfer. (b) aCGH result of an abnormal embryo

aneuploidy other than in the involved chromosomes in the sperms and embryos, which may be missed by FISH[10,11]. Undiagnosed aneuploidies may be able to explain the two early miscarriages in our patient when FISH was used for PGD.

A systematic review on the reproductive outcome in couples with translocation with recurrent pregnancy loss showed that the pregnancy rate was not improved after the use of PGD[12]. However, it was criticised that all studies in this review were using the FISH technique, with its known disadvantages as mentioned above. The negative results associated with FISH are confined not only to PGD for translocation carriers, but also extend to PGS. To date, there are 11 randomised controlled trials on the use of FISH for aneuploidy screening in early human embryos, showing no benefit in the pregnancy rate in specific groups of women, mostly those with advanced maternal age[13]. A position statement published by the European Society of Human Reproduction and Embryology[13] concluded that there was no evidence showing the beneficial effect with routine use of PGS for patients with advanced maternal age, and that

conclusive data on recurrent pregnancy loss, implantation failure, and severe male factor were missing.

There has been emerging evidence regarding the use of aCGH in both translocation carriers and preimplantation aneuploidy screening since 2008[4,7,14]. This technique is able to provide information on all 24 chromosomes for the detection of aneuploidy and translocation[7]. The use of aCGH combined with single blastocyst transfer was shown to produce promising results in patients with good prognosis and, in general, subfertile patients with improved pregnancy rates and reduced miscarriage rates[14,15]. It has largely replaced the role of FISH in both translocation and preimplantation aneuploidy screening. The protocol of using aCGH for single cell testing was launched in our unit in 2012, and now we have used this technique in 15 subjects for diagnosing both translocation carriers and PGS, with an ongoing pregnancy rate of 38.5% per transfer.

Conclusion

We report the first live birth in Hong Kong following PGD for translocation using aCGH. It reveals the feasibility and practicability of using aCGH in a single cell of PGD. Starting from 2013, we have replaced FISH-based PGD with the aCGH platform in our unit, which is also an emerging trend all over the world.

References

1. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990; 344:768-70.
2. Handyside AH, Lesko JG, Tarín JJ, Winston RM, Hughes MR. Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis. *N Engl J Med* 1992; 327:905-9.
3. Ng EH, Lau EY, Yeung WS, Lau ET, Tang MH, Ho PC. Pre-implantation genetic diagnosis in Hong Kong. *Hong Kong Med J* 2003; 9:43-7.
4. Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: microarrays and CGH. *Mol Hum Reprod* 2008; 14:703-10.
5. Voullaire L, Wilton L, McBain J, Callaghan T, Williamson R. Chromosome abnormalities identified by comparative genomic hybridization in embryos from women with repeated implantation failure. *Mol Hum Reprod* 2002; 8:1035-41.
6. Vanneste E, Voet T, Le Caignec C, et al. Chromosome instability is common in human cleavage-stage embryos. *Nat Med* 2009; 15:577-83.
7. Fiorentino F, Spizzichino L, Bono S, et al. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. *Hum Reprod* 2011; 26:1925-35.
8. Velilla E, Escudero T, Munné S. Blastomere fixation techniques and risk of misdiagnosis for preimplantation genetic diagnosis of aneuploidy. *Reprod Biomed Online* 2002; 4:210-7.
9. Li M, DeUgarte CM, Surrey M, Danzer H, DeCherney A, Hill DL. Fluorescence in situ hybridization reanalysis of day-6 human blastocysts diagnosed with aneuploidy on day 3. *Fertil Steril* 2005; 84:1395-400.
10. Pellestor F, Imbert I, Andréo B, Lefort G. Study of the occurrence of interchromosomal effect in spermatozoa of chromosomal rearrangement carriers by fluorescence in-situ hybridization and primed in-situ labelling techniques. *Hum Reprod* 2001; 16:1155-64.
11. Gianaroli L, Magli MC, Ferraretti AP, et al. Possible interchromosomal effect in embryos generated by gametes from translocation carriers. *Hum Reprod* 2002; 17:3201-7.
12. Franssen MT, Musters AM, van der Veen F, et al. Reproductive outcome after PGD in couples with recurrent miscarriage carrying a structural chromosome abnormality: a systematic review. *Hum Reprod Update* 2011; 17:467-75.
13. Harper J, Coonen E, De Rycke M, et al. What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium Steering Committee. *Hum Reprod* 2010; 25:821-3.
14. Forman EJ, Tao X, Ferry KM, Taylor D, Treff NR, Scott RT Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. *Hum Reprod* 2012; 27:1217-22.
15. Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. *Mol Cytogenet* 2012; 5:24.