J. Fetal Med. (March 2016) 3:25–30
DOI 10.1007/s40556-016-0078-y

ORIGINAL ARTICLE

First Successful Pregnancy After Pre-implantation Genetic Diagnosis by FISH for an Inversion Together with a Cryptic Translocation in India

Rupesh R. Sanap[1] · Arundhati S. Athalye[1] · Prochi F. Madon[1] · Nandkishor J. Naik[1] · Dattatray J. Naik[1] · Trupti V. Mehta[1] · Firuza R. Parikh[1]

Received: 26 August 2015 / Accepted: 9 February 2016 / Published online: 20 February 2016

Abstract The technique of pre-implantation genetic diagnosis (PGD) by fluorescence in situ hybridization (FISH) in cases of repeated miscarriages due to parental balanced inversions and translocations is relatively new in India. In a couple with a history of recurrent miscarriages and implantation failures, karyotyping done in three laboratories showed that the husband had an insertion or inversion of chromosome 12. Hence, they were referred to us for PGD. The anomaly turned out to be more complex. A pre-PGD workup using a series of FISH probes on metaphases accompanied by reflex FISH was required to characterize the anomaly. For subsequent PGD, single blastomeres were biopsied from seven embryos obtained by intracytoplasmic sperm injection. FISH analysis had to be carried out using ten probes in four rounds. On pre-PGD workup for inversion 12 by FISH, an additional anomaly of a cryptic translocation between 9qter and 12qter was detected in the husband. His complex karyotype according to the detailed ISCN nomenclature was therefore 46,XY,t(9;12)(9pter→9q34.1::12q24.2→12qter),der(12)-inv(12)(12pter→12p11.2::12q24.2→12p11.2::9q34.1→9qter). After PGD, the normal and balanced embryos transferred, resulted in the birth of healthy twins conceived in the first cycle itself. Therefore, a pre-PGD workup is important and needs reflex FISH in the event of an unexpected cytogenetic anomaly. PGD will need the analysis of additional chromosomes on the same cell by FISH in such cases. An experienced in vitro fertilization and Genetics team is essential for success. This is the first report of PGD by FISH for an inversion coupled with a cryptic translocation from India.

Keywords Pre-implantation genetic diagnosis · FISH · Inversion · Cryptic translocation · Pre-PGD workup · Reflex FISH · India

Introduction

Pre-implantation genetic diagnosis (PGD) is a technique where chromosome anomalies and single gene disorders can be checked in one or a few cells biopsied from embryos obtained during in vitro fertilization (IVF). Though this technique has been clinically used for around 25 years mainly in UK, USA, and Australia, it is a comparatively new field in India. For PGD of chromosomal aneuploidies and translocations, fluorescence in situ hybridization (FISH) was the standard method until a few years ago [1, 2]. Recent advances in PGD include comprehensive screening, enabled by trophectoderm biopsy and vitrification of biopsied blastocysts [3–7]. In India, very few centers have been successful in PGD by FISH [8–16], the first live births after PGD for Robertsonian and reciprocal translocations being reported in 2010 and 2014, respectively [17, 18]. This article illustrates the detection of an additional cryptic translocation during pre-PGD workup for an inversion. The husband was found to have a complex chromosomal anomaly necessitating the use of additional probes during PGD. The first PGD cycle was successful, leading to the birth of twins after a history of repeated miscarriages and implantation failures. This is the first report of live births after PGD for an inversion coupled with a cryptic translocation from India.

✉ Prochi F. Madon
prochi_madon@yahoo.com

[1] Department of Assisted Reproduction and Genetics, Jaslok Hospital and Research Center, 15, Dr. G. Deshmukh Marg, Mumbai, Maharashtra 400026, India

Materials and Methods

Clinical Data

The patients comprised a couple from a neighboring country who were referred after five cycles of intrauterine insemination (IUI) in their hometown resulted in two pregnancies, both of which aborted spontaneously, at 6 and 10 weeks. The husband had an impaired glucose tolerance for which he was given metformin hydrochloride while the 35-year-old wife had high CD19 and CD56 levels for which she was advised lymphocyte immune therapy. She was also on bromocriptinemesylate for hyperprolactinemia. Karyotyping done earlier in three different laboratories in India revealed a balanced structural anomaly on chromosome 12 in the husband. This was reported as 46,XY,ins(12)(q24.3p11.2p13) representing an insertion by two laboratories and 46,XY,der(12)inv(12)(p11.2q24.3) representing a pericentric inversion by another laboratory. The couple was given genetic counseling and referred to us in Mumbai for PGD. The wife was put on an antagonist protocol and started with a dose of 225 i.u. recombinant FSH with 225 i.u. HMG from day-two of menses. Inj. GnRh antagonist was added from day-six of stimulation. HCG trigger was given on day-ten of stimulation. Nine eggs (8 MII + 1 GV) were retrieved. Seven fertilized and cleaved. For PGD, a single blastomere was biopsied from each of the seven embryos after drilling the zona with a diode laser and tested by FISH after pre-PGD workup.

Pre-implantation Genetic Diagnosis Workup

FISH was carried out on metaphases and interphase nuclei of the couple obtained from PHA stimulated lymphocyte cultures, using Vysis (Abbott Molecular) or Kreatech probes for 12p (green), 12q (orange), and CEP 12 (aqua) to test the probes and check for signal polymorphisms. Subsequently, the BCR-ABL probe for 9q34 (orange), and 22q11.2 (green) was used, for confirmation of a cryptic translocation t(9;12)(q34;q24.3) suspected by metaphase FISH and inverted DAPI. This probe was readily available in our laboratory as it is routinely used to check for the Philadelphia chromosome in chronic myeloid leukemia (CML) caused by the reciprocal translocation t(9;22)(q34;q11.2). Hence, chromosome 22 was also checked simultaneously.

Pre-implantation Genetic Diagnosis

A single blastomere from each of the seven embryos was treated with hypotonic solution and fixed on slides with a 3:1 mixture of methanol and acetic acid, under a Nikon stereomicroscope. The nuclei were then observed under phase contrast of the Zeiss epi-fluorescent microscope Axioskop-2, and the co-ordinates were noted for relocation after hybridization and washing. FISH was carried out in four rounds on the same slides according to the manufacturers' short protocols. The BCR-ABL dual color dual fusion probe was used for a short hybridization time in round-one. After recording the results and capturing the images with the Metasystems Isis software, the slides were washed to strip the probes and a mixture of probes for 12p, 12q, and CEP 12 in green, orange, and aqua, respectively was used for overnight hybridization in round-two. For testing common aneuploidies, the AneuVysion CEP probe mixture, which is a combination of chromosomes X, Y, and 18, was used for a short hybridization in round-three and the Aneuvysion LSI probe mixture for chromosomes 13 and 21 was used for overnight hybridization in round-four. Thus, a total of ten probes (9q34, 22q11.2, 12p, 12q, CEP 12, CEP X, CEP Y, CEP 18, LSI 13, and LSI 21) were checked in 2 days for a day-five transfer. Institutional review board approval was taken for PGD.

Results

Pre-implantation Genetic Diagnosis Workup

FISH on fresh lymphocyte cultures of the couple with a mixture of probes for chromosome 12 in three colors confirmed inversion 12 in the husband, based on the difference in distance between the green (12p) and aqua (CEP 12) signals on the two homologues of chromosome 12 on metaphase spreads. The green and aqua signals were adjacent to each other on the normal 12, but were at two ends of the chromosome on the derivative 12 due to the pericentric inversion. However, it was also observed that the orange signal (12q), which should have been adjacent to the aqua signal on derivative 12, was not on the inverted 12, but on another medium-sized submetacentric chromosome (Fig. 1a). The inverted DAPI image of this metaphase suggested that the translocation was with 9q. Hence, reflex FISH was carried out on the same metaphase using the BCR-ABL cancer probe for CML, which was available in our laboratory. It showed that the orange signal for ABL (9q34) was located on derivative 12 adjacent to the centromere of chromosome 12 confirming a cryptic translocation t(9;12)(q34;q24.3), which was not detected earlier on karyotyping. Signal size polymorphism was also detected on pre-PGD workup, where one of the aqua signals for CEP 12 was much smaller in size (Fig. 1b). This small signal was found to be located on the centromere of the normal 12 in

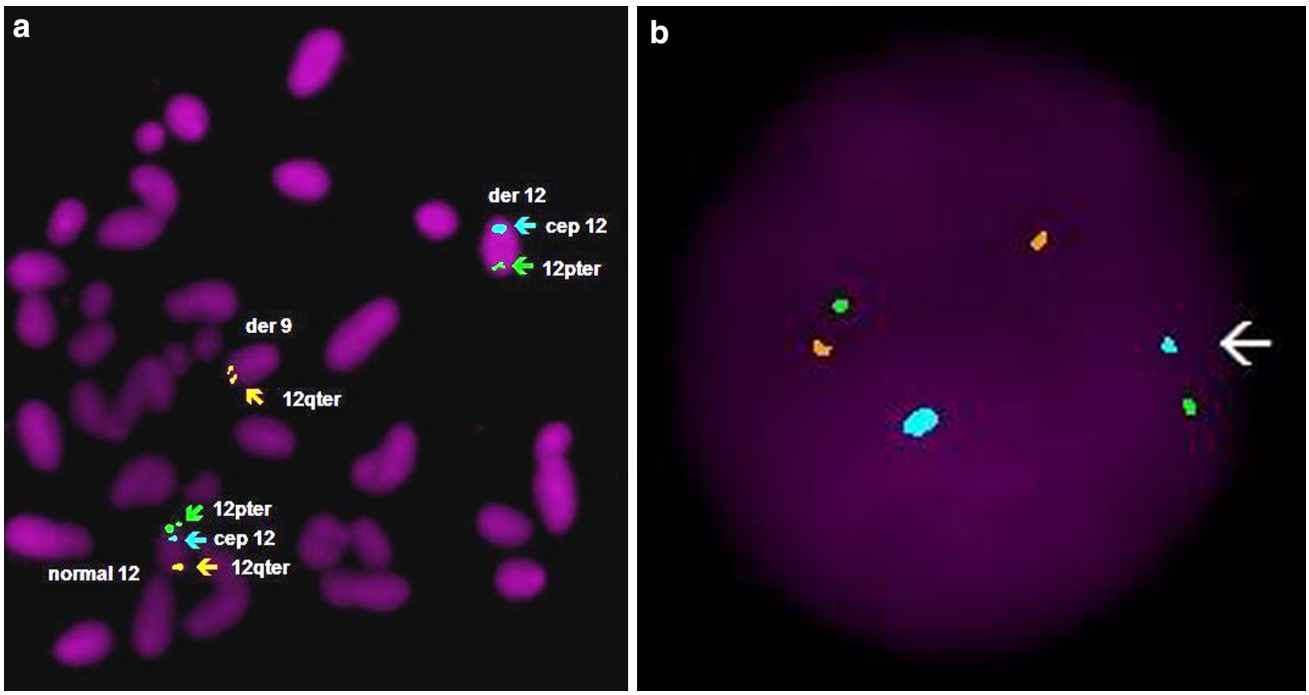


Fig. 1 **a** FISH on a metaphase cell of the husband, with a mixture of 12pter (*green*), CEP 12 (*aqua*), and 12qter (*orange*) showing inversion 12 together with a translocation of 12qter to a medium-sized submetacentric chromosome. **b** FISH on an interphase cell showing signal size polymorphism of the aqua signal for centromere 12. The small signal is marked with an *arrow*

metaphase spreads. The karyotype after pre-PGD workup was revised as follows: 46,XY,der(12)inv(12)(p11.2q24.2).ish inv(12),t(9;12)(q34.1;q24.2)(ABL1−,12qter+;12pter+,-cen12+,12qter−,ABL1+). According to the detailed ISCN nomenclature, the karyotype was designated as 46,XY,t(9;12)(9pter → 9q34.1::12q24.2 → 12qter),der(12)inv(12)(12pter → 12p11.2::12q24.2 → 12p11.2::9q34.1 → 9qter). A composite of chromosomes 9 and 12 from two G-banded metaphases at around 450 and 500 band resolutions is given in Fig. 2. In the present case, lymphocyte cultures were set up in our laboratory mainly to check the suitability of the FISH probes on metaphases and nuclei prior to PGD, hence banding of a higher resolution was not carried out.

Pre-implantation Genetic Diagnosis

PGD using FISH probes for 9q34 besides 12pter, 12qter, centromere 12, and common aneuploidies was carried out in four rounds on the same blastomeres. Of the single blastomeres biopsied from seven embryos, five were normal with no unbalanced translocation and no aneuploidy for the chromosomes tested, as they showed two signals for each probe used in four rounds (Fig. 3a–d). The corresponding normal embryos were transferred in the same cycle. Ultrasonography (USG) showed a twin pregnancy which resulted in the birth of healthy babies.

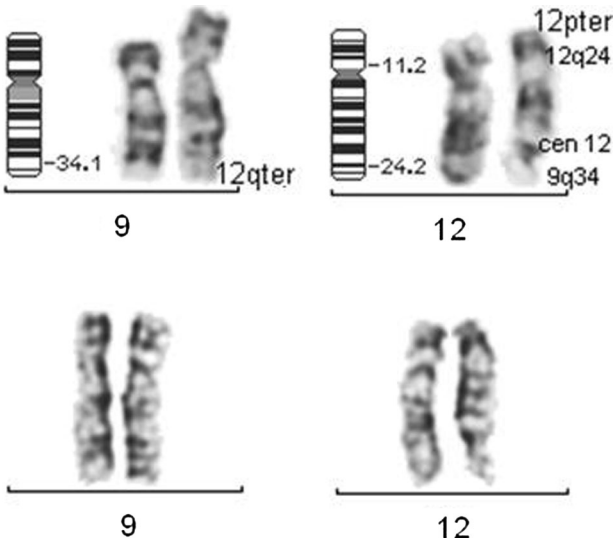
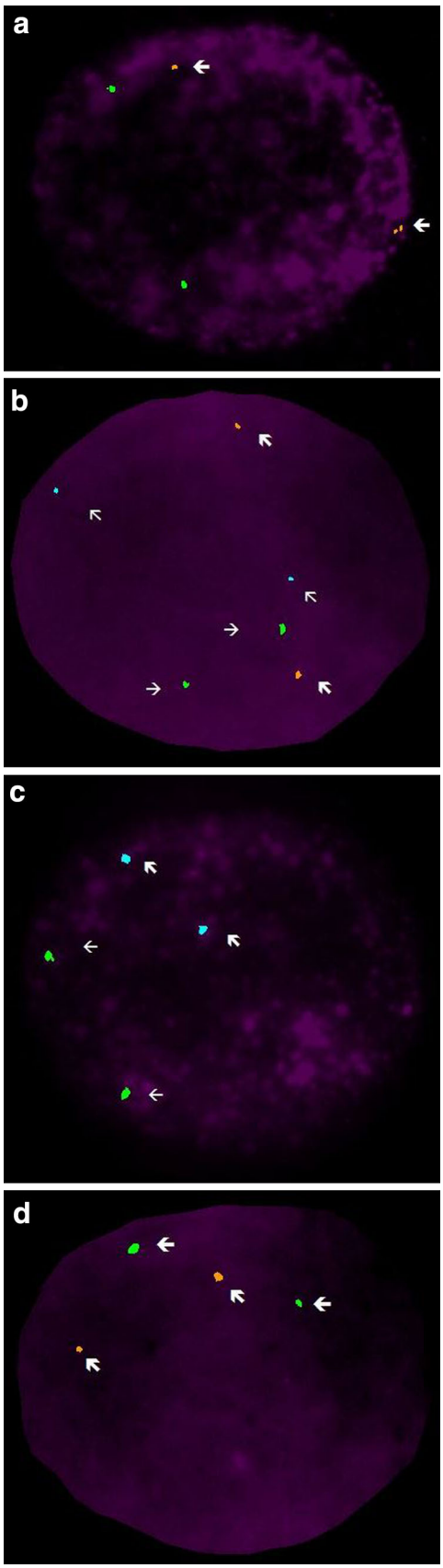


Fig. 2 Composite of chromosomes 9 and 12 in the husband, showing inversion 12 and a cryptic translocation between 9qter and 12qter

Genetic Counseling

The couple was given pre- and post-test genetic counseling. The cause of the recurrent miscarriages and the solution by PGD in their case was explained to them. They were aware that it would not be possible to differentiate between embryos carrying one or more balanced structural



◀**Fig. 3 a–d** FISH on a single blastomere in four rounds for PGD, showing two signals for each probe tested as described in the text, indicating an unaffected embryo suitable for transfer

anomalies, which were present in the husband and normal embryos without these anomalies, by FISH analysis on single cells. Only the unbalanced abnormal embryos would not be transferred in the IVF cycle. Though prenatal karyotyping is recommended even in the PGD reports, it has been observed that some couples are not willing to take the small risk involved in invasive prenatal procedures especially in precious pregnancies, with a previous bad obstetric history. Some of them are unwilling to test the carrier status of their children from cord blood at birth or during childhood, though they are aware that karyotyping will be necessary in adulthood prior to their children's marriage. In the present case, the couple was from an underdeveloped neighboring country and went back after embryo transfer. They informed us of the twin pregnancy detected on ultrasonography and of the birth of their children, though attempts to contact them regarding the karyotypes of the children failed. However, as the couple were educated, they were willing to guide their children on reproductive options later.

Discussion

PGD for aneuploidies by FISH in a clinical setting has been practiced for over two decades [19]. After success with aneuploidies, PGD by FISH for structural anomalies such as translocations and inversions was carried out [2, 20–23]. The European Society of Human Reproduction and Embryology (ESHRE) PGD consortium best practice guidelines for FISH-based PGD, were revised in 2010 [24]. It was recommended that prior to PGD, preliminary work on interphase nuclei and metaphase spreads obtained from peripheral blood lymphocytes of both reproductive partners, be carried out to check for signal polymorphisms or cross-hybridization, which is occasionally seen. In the present case, signal size polymorphism was observed for the centromere of chromosome 12 in the husband, where one of the two aqua signals was very small. The larger aqua signal was occasionally split and a faint connecting thread was observed between the split signals. This helped in interpretation of FISH results during PGD. Also, a cryptic translocation was picked up on metaphase spreads by FISH, substantiating the importance of detailed pre-PGD workup on interphase cells and metaphases. Prominent bands on chromosomes involved in constitutional and acquired translocations have also been observed.

According to the recent ESHRE PGD Consortium data collections XII and XIII for cycles between January 2009 and December 2010, out of 1882 cycles of PGD for chromosomal abnormalities, 93 (5 %) were for inversions, in which 117 embryos were transferred. A biochemical pregnancy was seen in 30 and a positive heartbeat in 24, resulting in 16 deliveries, with a live birth rate of 17.2 % for inversion cycles. There were two miscarriages while five clinical pregnancies were lost to follow up [25, 26]. The cycles for inversions and the number of deliveries was not given in earlier ESHRE PGD Consortium data I-XI [27]. A compilation of ESHRE PGD Consortium data I-XIII of 1832 cycles of PGD where the male partner was a carrier of a reciprocal translocation as in the present case, showed that 1779 embryos were transferred of which 412 showed a biochemical pregnancy and 310 had a positive heartbeat, giving a clinical pregnancy rate of 16.9 % for these cycles. The present case where PGD was carried out for a combination of an inversion and a translocation, resulted in the birth of twins in the first cycle itself.

Cryptic translocations are very small such as subtelomere translocations, which cannot be detected by karyotyping. In the present case, the cryptic translocation between the terminal regions of the long arms of chromosomes 9 and 12 could be detected by pre-PGD workup on metaphases of the husband, only because the inversion was also on chromosome 12 for which the probes were being tested. This suggests that there may be many idiopathic cases of recurrent miscarriages or repeated implantation failure where subtelomere testing by FISH on metaphases of the couple could pick up balanced chromosome rearrangements which are not detectable on karyotyping. A study on subtelomere FISH analysis of 11,688 individuals with developmental disabilities showed that approximately 60 % of the unbalanced translocations were inherited from a parent carrying a balanced form of the rearrangement [28]. Subtelomeres are known to contain a high concentration of genes as compared to other chromosome regions and they are very difficult, if not impossible, to detect by routine G-banding. Recent techniques such as array-CGH (comparative genomic hybridization) and MLPA (multiplex ligation probe amplification) can pick up genomic imbalances such as microduplications or microdeletions [29, 30]. However, balanced cryptic translocations or inversions which may be present in phenotypically normal couples with a history of recurrent pregnancy loss, cannot be detected with these techniques. This highlights the importance of subtelomeric FISH testing in selected cases.

The interface between assisted reproductive technologies and genetics is becoming more important with the increase in our understanding of the genetics of infertility [31]. As more genetic causes of reproductive failure are now recognized, the need for genetic counseling and PGD is increasing. This continually evolving field requires good communication between geneticists, IVF teams and patients to see that they are well informed while taking decisions [32].

Acknowledgments The authors would like to thank the entire staff of the Department of Assisted Reproduction and Genetics for their team work, and the management of Jaslok Hospital and Research Centre for supporting PGD initially through research grants RP 293 and RP 318.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to declare.

Informed Consent Informed consent was obtained from the couple.

Ethical Standard All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Munne S, Fragouli E, Colls P, Katz M, Schoolcraft W, Wells D. Improved detection of aneuploid blastocysts using a new 12 chromosome FISH test. Reprod Biomed Online. 2010;20:92–7.
2. Munne S. Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. Reprod Biomed Online. 2002;4:183–96.
3. Munne S. Preimplantation genetic diagnosis for aneuploidy and translocations using array comparative genomic hybridization. Curr Genomics. 2012;13:463–70.
4. Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive screening at the blastocyst stage. FertilSteril. 2010;94:1700–6.
5. Wu MY, Chao KH, Chen CJ, Chang LJ, Chen SU, Yang YS. Current status of comprehensive chromosome screening for elective single-embryo transfer. Obstet Gynecol Int. 2014;. doi:10.1155/2014/581783.
6. Fragouli E, Alfarawati S, Daphnis DD, GoodallNN MariaA, Griffiths T, et al. Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH, scientific data and technical evaluation. Hum Reprod. 2011;26:480–90.
7. Alfarwati S, Fragouli E, Colls P, Wells D. First births after preimplantation genetic diagnosis of structural chromosomal abnormalities using comparative genomic hybridization and microarray analysis. Hum Reprod. 2011;26:1560–74.
8. Parikh FR, Madon PF, Athalye AS, Naik NJ, Gada SD, Ganla KN, et al. Preimplantation genetic diagnosis of chromosome abnormalities by multicolour fluorescence in situ hybridization. J Indian Med Assoc. 2001;99:441–4.
9. Madon PF, Athalye AS, Naik NJ, Parikh FR. Preimplantation genetic diagnosis. In: Telang M, editor. Atlas of human assisted reproductive technologies. Delhi: Jaypee; 2007. p. 167–74.
10. Parikh F, Naik N, Gada S, Bhartiya A, Athalye A, Madon P. Preimplantation genetic diagnosis for the better management of couples during assisted reproduction. Int J Hum Genet. 2001;1:117–21.
11. Athalye AS, Madon PF, Parikh FR. Preimplantation genetic diagnosis: Indian experience. Int J Hum Genet. 2006;6(Suppl 2):38.

12. Madon PF. Conventional and molecular cytogenetics in India. In: Kumar D, editor. Genomics and health in the developing world. Oxford (medical monographs); 2012. p. 1096–111.
13. Sharma S, Sharma R. Preimplantation genetic diagnosis. In: Talwar P, editor. Manual of assisted reproductive technologies and clinical embryology. Jaypee Brothers, 2012:327–33.
14. GadaSaxena S, Desai K, Shewale L, Ranjan P. Pre-implantation genetic screening using fluorescence in situ hybridization in couples of Indian ethnicity: Is there a scope? J Hum Reprod Sci. 2014;7:25–9.
15. Madon P. Challenges in prenatal and pre-implantation genetic diagnosis studies. Mol Cytogenet. 2014;7(Suppl 1):150. doi:10.1186/1755-8166-7-S1-I50.
16. Madon P, Athalye A, Sanap R, Naik D, Naik N, Parikh R. Preimplantation genetic diagnosis by FISH in India: the Jaslok Hospital experience. Prepr Cytogenet Genome Res. 2014;142:226. doi:10.1159/000360710.
17. Madon PF, Athalye AS, Naik NJ, Naik DJ, Parikh FR. PGD for a Robertsonian translocation by FISH: first successful pregnancy from India. J Prenat Diagn Ther. 2010;1:20–2.
18. Naik DJ, Madon PF, Naik NJ, Athalye AS, Parikh FR. PGD by FISH for a reciprocal translocation: first baby from India. J Fetal Med. 2014;1:41–3.
19. Harper JC, Delhanty JD, Handyside AH, editors. Preimplantation genetic diagnosis. New York: Wiley; 2003.
20. Escudero T, Lee M, Stevens J, Sandalinas M, Munne S. Preimplantation genetic diagnosis of pericentric inversions. Prenat Diagn. 2001;21:760–6.
21. Iwarson E, Ahrlund-Richter L, Inzunza J, Rosenlund B, Fridstrom M, Hillensjo T, et al. Preimplantation genetic diagnosis of a large pericentric inversion of chromosome 5. Mol Hum Reprod. 1998;4:719–23.
22. Wiland E, Hobel CJ, Hill D, Kurpisz M. Successful pregnancy after preimplantation genetic diagnosis for carrier of t(2;7)(p11.2,q22) with high rates of unbalanced sperm and embryos: a case report. Prenat Diagn. 2008;28:36–41.
23. Van Assche E, Staessen C, Vegetti W, Bonduelle M, Vandervorst M, Van Steirteghem A, et al. Preimplantation genetic diagnosis and sperm analysis by fluorescence in situ hybridization for the most common reciprocal translocation t(11;22). Mol Hum Reprod. 1999;5:682–90.
24. Harton GL, Harper JC, Coonen E, Pehlivan T, Vesela K, Wilton L. ESHRE PGD consortium best practice guidelines for fluorescence in situ hybridization-based PGD. Hum Reprod. 2010;00:1–8.
25. Moutou C, Goossens V, Coonen E, De Rycke E, Kokkali G, Renwick P, et al. ESHRE PGD consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010. Hum Reprod. 2014;29:880–903.
26. De Rycke M, Belva F, Goossens V, Moutou C, SenGupta S, Traeger-Synodinos J, Coonen E. ESHRE PGD consortium data collection XIII: cycles from January to December 2010 with pregnancy follow-up to October 2011. Hum Reprod. 2015;2015(30):1763–89.
27. Goossens V, Traeger-Synodinos J, Coonen E, De Rycke M, Moutou C, Pehlivan T, et al. ESHRE PGD consortium data collection XI: cycles from January to December 2008 with pregnancy follow-up to October 2009. Hum Reprod. 2012;27:1887–911.
28. RavnanJB TepperbergJH, Papenhausen P, Lamb AN, Hedrick J, Eash D, et al. Subtelomere FISH analysis of 11688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. J Med Genet. 2006;43:478–89.
29. Le Caignec C, Boceno M, Saugier-Veber P, Jacquemont S, Joubert M, David A, et al. Detection of genomic imbalances by array based comparative genome hybridization in fetuses with multiple malformations. J Med Genet. 2005;42:121–8.
30. Goumy C, Gouas L, Pebrel-Richard C, Veronese L, Eymard-Pierre E, Debost-Legrand A, et al. Prenatal detection of cryptic rearrangements by multiplex ligation probe amplification in fetuses with ultrasound abnormalities. Genet Med. 2010;12:376–80.
31. Geraedts J. Reproductive genetics at the crossroads of the European Society of Human Reproduction and Embryology and the European Society of Human Genetics: an update. Hum Reprod. 2014;29:1601–2.
32. Harper J, Geraedts J, Borry P, Cornel MC, DondorpWJ GianaroliL, et al. Current issues in medically assisted reproduction and genetics in Europe: research, clinical practice, ethics, legal issues and policy. Hum Reprod. 2014;29:1603–9.