Pre-implantation genetic screening using fluorescence *in situ* hybridization in couples of Indian ethnicity: Is there a scope?

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ABSTRACT

CONTEXT: There is a high incidence of numerical chromosomal aberration in couples with repeated *in vitro* fertilization (IVF) failure, advanced maternal age, repeated unexplained abortions, severe male factor infertility and unexplained infertility. Pre-implantation genetic screening (PGS), a variant of pre-implantation genetic diagnosis, screens numerical chromosomal aberrations in couples with normal karyotype, experiencing poor reproductive outcome. The present study includes the results of the initial pilot study on 9 couples who underwent 10 PGS cycles. **AIM:** The aim of the present study was to evaluate the beneficial effects of PGS in couples with poor reproductive outcome. **SETTINGS AND DESIGN:** Data of initial 9 couples who underwent 10 PGS for various indications was evaluated. **SUBJECTS AND METHODS:** Blastomere biopsy was performed on cleavage stage embryos and subjected to two round fluorescence *in situ* hybridization (FISH) testing for chromosomes 13, 18, 21, X and Y as a two-step procedure. **RESULTS:** Six of the 9 couples (10 PGS cycles) conceived, including a twin pregnancy in a couple with male factor infertility, singleton pregnancies in a couple with secondary infertility, in three couples with adverse obstetric outcome in earlier pregnancies and in one couple with repeated IVF failure. **CONCLUSION:** In the absence of availability of array-comparative genomic hybridization in diagnostic clinical scenario for PGS and promising results with FISH based PGS as evident from the current pilot study, it is imperative to offer the best available services in the present scenario for better pregnancy outcome for patients.

KEY WORDS: Aneuploidy testing, fluorescence *in situ* hybridization, genetic counseling, pre-implantation genetic diagnosis, pre-implantation genetic screening

INTRODUCTION

An estimated total of 5 million babies have been born world-wide, since the birth of the first baby Louise Joy Brown in 1978 through *in vitro* fertilization (IVF). As per the European Society of Human Reproduction and Embryology press released,[1] there are approximately 1.5 million assisted reproduction technologies cycles globally annually, resulting in about 350,000 babies. India did not lag behind in the field of IVF and the first Indian IVF baby was born, just 3 months after Louise Brown.[2] As per the survey by outlook business magazine (2011),[3] there are approximately 90,000 IVF cycles performed in India alone with approximately 20,000 children born annually.

There have been tremendous advances in the field of assisted reproduction to enhance the success rate including methods to optimize embryo selection. Studies have shown a high incidence of numerical chromosomal aberration in couples with repeated IVF failure, advanced maternal age, repeated unexplained abortions.[4-6] Embryo selection based on morphological evaluation either on day 3 or day 5 of development does not ensure a normal chromosomal constitution[6,7] and this may be the cause of low implantation potential. Pre-implantation genetic diagnosis (PGD) is a recent diagnostic modality, referring to procedures performed to identify genetic defects, in embryos generated *in vitro*, prior to implantation.[8] Pre-implantation genetic screening (PGS),

a variant of PGD, screens numerical chromosomal aberrations in couples with normal karyotype, experiencing poor reproductive outcome including advanced maternal age, repeated IVF failures, repeated spontaneous abortions, severe male factor infertility and unexplained infertility. PGS is offered by many IVF centers to improve the reproductive outcome of the specific group of patients.[9] PGS thus brings the hope of healthy babies to couples at risk of passing heritable diseases to their offspring. PGS diagnoses the genetic aberrations at the pre-implantation stage, i.e. before implantation,[10] thus helping in negative selection of the affected embryos prior to implantation. PGS can be performed at different stages of embryo development including polar body biopsy, blastomere biopsy from cleavage stage embryos, trophectoderm biopsy from blastocyst. The diagnosis of monogenic disorders is done using the polymerase chain reaction based technique, whereas fluorescence *in situ* hybridization (FISH) can be used for PGS as well as diagnosis of numerical and structural chromosomal aberrations.

FISH is a molecular cytogenetic technique for numerical constitutional chromosomal picture of an embryo, to provide results within the time frame of IVF procedures. The cells, including polar body, blastomere or trophectoderm cells, are fixed on glass microscope slides and fluorescently labeled deoxyribonucleic acid (DNA) probe hybridized to an *in situ* chromosomal target. The probes are targeted to specific regions or genes in the chromosome and are labeled with fluorochromes. Currently, a panel of probes is commercially available for different segments of the chromosomes. However, a limited number of fluorochromes are used as labels, confining the number of chromosomes that can be analyzed simultaneously. Probe detection is accomplished by ultraviolet light excitement of a fluorochrome. Aneuploidies for chromosomes 13, 18, 21, X and Y account for 90-95% of chromosomal aberrations in live-born infants. Hence, in the present study, we perform FISH only for chromosomes 13, 18, 21 and sex chromosomes. Furthermore, increasing the number of chromosomes would increase the cost which is not always acceptable by the referring IVF specialist.

There are few centers in India offering PGD/PGS. Our laboratory is a satellite referral laboratory, where we offer PGD/PGS to various centers across India. Personnel from our team visit the IVF center on day-3 post fertilization for embryo biopsy and blastomere fixation and the slides are brought back for FISH testing on the same day. The locus specific identifier (LSI) 13 SpectrumGreen and LSI 21 SpectrumOrange probes were used to detect aneuploidies of chromosomes 13 and 21; and chromosome enumeration probe (CEP) 18 SpectrumAqua, X SpectrumGreen and Y SpectrumOrange probes were used to detect aneuploidies of chromosomes 18, X and Y. FISH is performed as a two-step procedure and the results are released by day-4 evening and hence that the embryo transfer can be performed in the same IVF cycle on day-5 at the blastocyst stage. In the present report, we present the data of the initial 10 PGS cycles performed in 9 couples for various clinical indications including male factor infertility ($n = 1$), recurrent spontaneous abortions (RSAs) ($n = 4$), repeated IVF failure ($n = 3$), secondary infertility ($n = 1$). One of the patients underwent two cycles of PGS [P6 and P7 in Table 1]. All these couples had normal karyotype.

MATERIALS AND METHODS

The patients underwent various stages of IVF cycle including superovulation, oocyte retrieval, *in vitro* embryo culture using standard protocols. Oocyte retrieval was carried out 34-36 h after administration of injectable human chorionic

Table 1: Summary of the 10 PGD cycles showing the clinical indication for PGS, the details of a number of embryos biopsied and FISH results and pregnancy outcome

Patient	Clinical indication**	No. of embryos	No. results	Euploid	T 21	T 13	T 18	Monosomy X	Triploidy	PGD outcome
P1	Male factor infertility	17	1	10	2	1	2	1	-	Pregnant
P2	Secondary infertility	6	-	4	1	-	1	-	-	Pregnant
P3	RSA	5	-	3	1	-	1	-	-	Pregnant
P4	RSA	11	-	8	1	1	-	-	1	Pregnancy in frozen transfer cycle
P5	RSA	1	-	1	-	-	-	-	-	-
P6*	RSA	2	-	-	2	-	-	-	-	No transfer
P7*	RSA	2	-	2	-	-	-	-	-	Pregnant
P8	Repeated IVF failure	10	1	6	2	-	1	-	-	-
P9	Repeated IVF failure	5	2	1	-	1	-	1	-	Pregnant
P10	Repeated IVF failure	9	-	8	1	-	-	-	-	-
Total		68	4	43	10	3	5	2	1	6

T 21=Trisomy 21, T 13=Trisoy 13, T 18=Trisomy 18, RSA=Recurrent spontaneous abortion, *P6 and P7=Same patients who had two PGS cycles, **Clinical indications, P1=Hypogonadotropic hypopituitarism male infertility, P2=Secondary infertility of unknown etiology, P3 and P6=Three first trimester spontaneous abortions, P4=Four first trimester spontaneous abortions, P5=Six first trimester spontaneous abortions, P8=Four failed IVF cycles, P9=Three failed IVF cycles, P10=Six failed IVF cycles, PGS=Pre-implantation genetic screening, IVF=*In vitro* fertilization, PGD=Pre-implantation genetic diagnosis, FISH=Fluorescence *in situ* hybridization

gonadotropin by ultrasound guided follicular aspiration. Mature oocytes were subjected to intracytoplasmic sperm injection followed by *in vitro* embryo culture in the incubator at 37°C and 5% CO_2. Cleavage stage embryo biopsy on day 3 embryos at 6-10 cell of development was performed using non-contact Saturn 3™ laser system (Research Instruments, Cornwall, UK), followed by aspiration of single blastomere from each embryo. The fundamental criterion for the selection of the blastomere was the presence of a clear nucleus and maintenance of cell integrity. The embryos were manipulated singly to reduce the amount of time spent outside the incubator.

The blastomere was fixed immediately post-biopsy. The blastomere was exposed to hypotonic solution (0.075 mol/l potassium chloride supplemented with 0.6% bovine serum albumin [w/v]) for 2 min. The blastomere was then placed onto the microscope slide in a small hypotonic drop using 80 µ flexipet (Cook, Australia). A drop of chilled methanol: acetic acid (3:1) fixative was added over the blastomere, which resulted in bursting of the cytoplasm and fixation of blastomere nucleus on the slide. The nucleus was identified under the microscope and labeled with the embryo number. Approximately, 4-6 blastomeres were fixed on each slide and minimum two slides were prepared per patient. The fixed slides were then transported to the laboratory in slide box at room temperature for FISH testing. The slides were dehydrated by passing through alcohol grades (70% ethanol w/v: 1 min; 85% ethanol w/v: 1 min and 100% ethanol: 2 min). FISH was carried out as a two-step procedure. In the first round, FISH testing was carried out for chromosomes 13 and 21 and then for chromosomes 18, X and Y. 3 µl probe for chromosome 13 and 21 using LSI 13 SpectrumGreen/21 SpectrumOrange probe (Abbott, USA) was applied on the coverslip and the slide was inverted on the coverslip. The probe and nuclear DNA were denatured simultaneously at 73°C for 8-10 min and left to hybridize at 37°C overnight. To remove unbound probe, the slides were washed in wash solutions containing 0.3% Igepal in 0.4X SSC for 2 min at 73°C followed by 0.1% Igepal in 2X SSC for 1 min at room temperature. The slides were mounted in Vectarshield (Vector Laboratories, CA, USA) antifade medium containing 200 ng DAPI/ml to counterstain the nuclei and examined under Olympus AX70 microscope using appropriate filters with Cytovision software (Olympus, USA). Appropriate controls were included in the test. After analysis, the slides were washed under running tap water to wash off the probe. The slides were again dehydrated using alcohol grades and then subjected to FISH for chromosome 18, X and Y using CEP 18 SpectrumAqua/X SpectrumGreen/Y SpectrumOrange probe (Abbott, USA) following the same protocol.

Blastomere was labeled as normal diploid when two signals for each of the fluorochrosome were observed. Presence of three signals for any of the fluorochromes indicated trisomy and presence of one signal indicated monosomy for that chromosome.

RESULTS

In the present report, 10 PGS cycles performed in 9 couples for various clinical indications including male factor infertility (n = 1), RSAs (n = 4), repeated IVF failure (n = 3), secondary infertility (n = 1). The details of the number of embryos biopsied, the FISH results and the pregnancy outcome are listed in Table 1.

In the couple with male factor infertility due to hypogonadotropic hypopituitarism (P1), 19 oocytes were retrieved of which 17 fertilized and were at 6-8 cell stage of development on day-3. Embryo biopsy was performed on all 17 embryos. Blastomere from one embryo lysed during biopsy and since the embryo was grade III embryo with a lot of fragmentation, second blastomere was not biopsied. FISH was performed for chromosomes 13, 18, 21, X and Y. Trisomy 21 was detected in 2 embryos, trisomy 18 in 2 embryos, trisomy 13 in 1 embryo and monosomy X in one embryos. Three of the 10 euploid embryos were transferred on day-5 at the blastocyst stage and in resulted in a twin pregnancy.

Another couple (P2), a 32-year-old female and 35-year-old male married for 9 years presented with secondary infertility (etiology unknown) following birth of a male child 8 years back who died at the age of 2 months due to sudden infant death syndrome. Keen to have a child, the couple underwent IVF/PGS cycle. Of the 10 oocytes retrieved, 8 fertilized and 6 embryos were at 6-8 cell stage of development on day 3 post-insemination. Embryo biopsy and FISH performed on all 6 embryos, revealed trisomy 21 in one embryo and trisomy 18 in one embryo. Two of the 4 euploid embryos were transferred and it resulted in a singleton pregnancy.

Of the 5 couples referred for PGS following adverse obstetric outcome in earlier pregnancies, two couples conceived in the same cycle and one patient conceived following frozen-thawed embryo transfer and all three patients delivered normal healthy babies at term. Another couple (P4), a 36-year-old female and 41-year-old male married for 10 years with a history of 4 spontaneous first trimester abortions. Of the 19 oocytes retrieved, 15 fertilized and 11 embryos were at 6-8 cell stage of development on day 3 post-insemination. Embryo biopsy and FISH performed on all 11 embryos, revealed triploidy in one embryo, trisomy 21 in one and trisomy 13 in one embryo. Due to some personal unavoidable reasons, the female did not have any embryo transfer in the same cycle. All the 8 euploid embryos were frozen at the blastocyst stage and two blastocysts were thawed in the subsequent cycle and resulted in a singleton

pregnancy. One of the three patients with repeated IVF failure conceived and delivered one normal healthy baby at term. The details of the number of embryos biopsied and the FISH results are listed in Table 1. While releasing the patient report, as the Pre-natal Diagnostic Techniques (Regulation and Prevention of Misuse) Act, 1994, the results of the sex chromosome are masked.

Of the 68 embryos biopsied in the 10 PGS cycles, no results were obtained in 4 embryos. This was due to lysis of the blastomere during biopsy in two embryos, fragmentation of blastomere in during fixing in one embryo and loss of blastomere during blastomere fixation. Of the 64 blastomere subjected to FISH testing, 43 blastomere (67.18%) were found to be euploid for chromosome 13, 18, 21, X and Y. Trisomy 21 was the most common aneuploidy detected in 10 embryos (15.62%), followed by trisomy 18 in 5 embryos (7.81%), trisomy 13 in 3 embryos (4.69%), monosomy X in 2 embryos and triploidy in one embryos. Ten of the 21 aneuploid embryos were available for re-analysis on day-5. All the cells were biopsied in these embryos and subjected to FISH testing. The results of FISH testing were in concordance with the earlier results except in one embryo with trisomy 21, which was found to be mosaic.

All the six pregnant patients had prenatal diagnosis by amniocentesis and FISH/karyotyping testing performed elsewhere and the fetus were found to be normal. All the six patients have delivered normal healthy children at term.

DISCUSSION

Aneuploidies of chromosome 13, 18, 21, X and Y constitutes 90-95% of chromosomal aberrations in live-born infants. Hence, at present we offer PGS for these 5 chromosomes. Many centers offering PGS analyze between 8-12 chromosomes. With an increasing number of chromosomes analyzed, lower efficiency per probe has been reported.[11,12]

There have been multiple controversial prospective randomized controls trials questioning the usefulness of FISH based PGD/PGS. Mastenbroek *et al*.,[13] Hardarson *et al*.,[14] and Jansen *et al*.,[15] in their study have reported statistically detrimental results in a live birth rates in PGS group compared with the control group. Staessen *et al*.,[6] Blockeel *et al*.,[16] Debrock *et al*.,[17] observed no statistical differences between the two groups; whereas Werlin *et al*., 2003[18] and Schoolcraft *et al*.,[19] in their study observed potential benefits of PGS. However, these poor outcomes may be related to high dexterity required for performing embryo biopsy and blastomere fixation and should be performed by experienced personnel. In the absence of trained embryologist performing embryo biopsy procedure, the survival and potential implantation of the embryo may get affected.[20] Blastomere fixation is the crux of the entire procedure. Improper fixation can result in poor and non-informative FISH results, making diagnosis difficult.[21,22] In addition, FISH results need to be interpreted with caution, especially in case of overlapping signals, split signals, weak and faded signals.[23,24]

With PGS, selection of euploid embryos for transfer helps to improves implantation and pregnancy rate and a reduced miscarriage rate. In the present report, of the 10 PGS cycles performed for various indications, pregnancy was achieved in 6 patients. Aneuploidy was observed in 32.82% embryos subjected to embryo biopsy and FISH testing. Greater than 50% of these aneuploid embryos had reached the blastocyst stage. In the absence of PGS, if these embryos had been selected for transfer based on the morphology, then the chances of implantation and subsequent pregnancy would have reduced with an increased chance of first trimester abortion. There has been an ongoing debate over the benefits of PGD/PGS world-wide. However, in our experience, PGS has shown to be beneficial in this selected group of patients.

Because the testing is only done on one or two cells from a single embryo, there is a possibility that the cells tested do not accurately represent the status of the entire embryo. Studies have shown that PGS is usually about 85% accurate, with the potential for both false-positive or false-negative results. Hence, it is recommended to confirm the results of PGS on prenatal diagnosis by amniocentesis or chorionic villus sampling testing.

Recent advances in the field of molecular biology are aiming toward developing technique allowing 24-chromosome analysis and also provide results in the time frame of an IVF cycle. These include combination of comparative genomic hybridization (CGH) combined with microarrays, offering better resolution than conventional CGH. Further research on CGH and array-CHG have improved the robustness and accuracy of the technique making it a promising technique for PGS aneuploidy screening.

The first Indian IVF baby was born just 3 months after the world's first IVF baby. However, the Indian sub-continent has failed to keep pace with the developed countries in the field of genetic testing and PGD/PGS. There is a visible lacunae that currently exists in the diagnostic services on the Indian scene and PGD/PGS tests are not available in Pathological, Academic, Research or Referral Laboratories in India. To fill this void present, our laboratory has been operating as a satellite referral laboratory for PGD/PGS both of aneuploidy screening as well as monogenic disorders with the first reported pregnancy for PGD for β-thalassemia in India.[25] However, there are no commercial laboratories including ours, which offers array-CGH as a clinical service to patient populations for

PGD/PGS. The initial results on these 10 cases reported in the present study, demonstrated a beneficial effect of PGS using FISH based technology. Following this additional 21 patients have undergone PGS for aneuploidy screening (data not included), with 10 pregnancies, including 1 twin pregnancy, showing favorable outcome for PGS. It is thus imperative to integrate basic genetic services into the existing primary health care and medical services including karyotyping in couples with reproductive failure,[26] PGS using FISH in the absence of availability of microarray based technology for PGS and community control of common genetic aberrations as priority needs for biomedical intervention.

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REFERENCES

1. Available from: http://www.eshre.eu/Press-Room/Press-releases/Press-releases-ESHRE-2012/5-million-babies.aspx. [Last accessed on 2014 Mar 03].
2. Chaterjee S. Prof. Robert Edwards, Nobel Laureate in medicine 2010: Tribute of an Indian with joy and sorrow. Editorial. Al Ameen J Med Sci 2011;4:1-2.
3. Available from: http://www.business.outlookindia.com/printarticle.aspx?278530. [Last accessed on 2014 Mar 03].
4. Gianaroli L, Magli MC, Munné S, Fiorentino A, Montanaro N, Ferraretti AP. Will preimplantation genetic diagnosis assist patients with a poor prognosis to achieve pregnancy? Hum Reprod 1997;12:1762-7.
5. Gianaroli L, Magli MC, Ferraretti AP, Munné S. Preimplantation diagnosis for aneuploidies in patients undergoing *in vitro* fertilization with a poor prognosis: Identification of the categories for which it should be proposed. Fertil Steril 1999;72:837-44.
6. Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, *et al*. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: A prospective randomized controlled trial. Hum Reprod 2004;19:2849-58.
7. 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.
8. Sermon K. Current concepts in preimplantation genetic diagnosis (PGD): A molecular biologist's view. Hum Reprod Update 2002;8:11-20.
9. Rubio C, Bellver J, Rodrigo L, Bosch E, Mercader A, Vidal C, *et al*. Preimplantation genetic screening using fluorescence *in situ* hybridization in patients with repetitive implantation failure and advanced maternal age: Two randomized trials. Fertil Steril 2013;99:1400-7.
10. Egozcue J, Santaló J, Giménez C, Pérez N, Vidal F. Preimplantation genetic diagnosis. Mol Cell Endocrinol 2000;166:21-5.
11. Abdelhadi I, Colls P, Sandalinas M, Escudero T, Munné S. Preimplantation genetic diagnosis of numerical abnormalities for 13 chromosomes. Reprod Biomed Online 2003;6:226-31.
12. Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, *et al*. Milder ovarian stimulation for *in-vitro* fertilization reduces aneuploidy in the human preimplantation embryo: A randomized controlled trial. Hum Reprod 2007;22:980-8.
13. Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, *et al*. *In vitro* fertilization with preimplantation genetic screening. N Engl J Med 2007;357:9-17.
14. Hardarson T, Hanson C, Lundin K, Hillensjö T, Nilsson L, Stevic J, *et al*. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: A randomized controlled trial. Hum Reprod 2008;23:2806-12.
15. Jansen RP, Bowman MC, de Boer KA, Leigh DA, Lieberman DB, McArthur SJ. What next for preimplantation genetic screening (PGS)? Experience with blastocyst biopsy and testing for aneuploidy. Hum Reprod 2008;23:1476-8.
16. Blockeel C, Schutyser V, De Vos A, Verpoest W, De Vos M, Staessen C, *et al*. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. Reprod Biomed Online 2008;17:848-54.
17. Debrock S, Spiessens C, Peeraer K, De Loecker P, Willemen D, D'Hooghe TM. Higher implantation rate using modified quarter laser-assisted zona thinning in repeated implantation failure. Gynecol Obstet Invest 2009;67:127-33.
18. Werlin L, Rodi I, DeCherney A, Marello E, Hill D, Munné S. Preimplantation genetic diagnosis as both a therapeutic and diagnostic tool in assisted reproductive technology. Fertil Steril 2003;80:467-8.
19. Schoolcraft WB, Katz-Jaffe MG, Stevens J, Rawlins M, Munne S. Preimplantation aneuploidy testing for infertile patients of advanced maternal age: A randomized prospective trial. Fertil Steril 2009;92:157-62.
20. Simpson JL. What next for preimplantation genetic screening? Randomized clinical trial in assessing PGS: Necessary but not sufficient. Hum Reprod 2008;23:2179-81.
21. Munné S, Dailey T, Finkelstein M, Weier HU. Reduction in signal overlap results in increased FISH efficiency: Implications for preimplantation genetic diagnosis. J Assist Reprod Genet 1996;13:149-56.
22. 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.
23. Magli MC, Sandalinas M, Escudero T, Morrison L, Ferraretti AP, Gianaroli L, *et al*. Double locus analysis of chromosome 21 for preimplantation genetic diagnosis of aneuploidy. Prenat Diagn 2001;21:1080-5.
24. Colls P, Escudero T, Cekleniak N, Sadowy S, Cohen J, Munné S. Increased efficiency of preimplantation genetic diagnosis for infertility using "no result rescue". Fertil Steril 2007;88:53-61.
25. Gada Saxena S, Saranath D. Single-cell polymerase chain reaction-based pre-implantation genetic diagnosis using fragment analysis for β-thalassemia in an Indian couple with β-globin gene mutations. J Hum Reprod Sci 2012;5:289-92.
26. Gada Saxena S, Desai K, Shewale L, Ranjan P, Saranath D. Chromosomal aberrations in 2000 couples of Indian ethnicity with reproductive failure. Reprod Biomed Online 2012;25:209-18.

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