

Successful birth of South India's first twins after preimplantation genetic screening of embryos

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ABSTRACT

We report the first documented successful birth of twins following preimplantation genetic screening (PGS) of cleavage stage embryos by array comparative genomic hybridization (CGH) technology, in South India. The case was a 28-year-old woman with the previous history of preclinical pregnancy and a miscarriage in two attempted *in vitro* fertilization cycles. Day 3 cleavage stage embryos were generated by conventional long protocol with the use of a gonadotropin-releasing hormone analog and a combination of recombinant folliculotropins and human menopausal gonadotropins. Intracytoplasmic sperm injection of oocytes thus obtained was performed, and 10 selected embryos underwent PGS using the array CGH technique. Two normal blastocysts were transferred to the patient, and she conceived twins. She delivered at 35 weeks of gestation by elective cesarean on November 19, 2014. She delivered a healthy male and female baby weighing 2.19 kg and 2.26 kg, respectively. Postnatal evaluation of babies was also normal, and the hospital course was uneventful. PGS has a definitive indication in assisted reproductive technology programs and can be utilized to improve pregnancy rates significantly.

KEY WORDS: Abnormal embryos, embryo biopsy, genetic screening, preimplantation genetic screening

INTRODUCTION

The field of assisted reproduction has been a witness to emerging new trends in laboratory techniques since the past two decades. One such arena is preimplantation genetic screening (PGS) of embryos. As opposed to genetic diagnosis, which is more specialized and restrictive, simple screening of embryos for chromosomal normalcy is more applicable in conventional clinical practice as a means to significantly improve pregnancy rates in assisted reproductive technology (ART) programs. It has been well-documented that at least 60% of embryos generated by various laboratory procedures used in conventional *in vitro* fertilization (IVF) protocols are chromosomally incompetent.^[1] The errors leading to numerical abnormalities in these embryos are responsible for the current success rates in ART.

We herewith present a case report where the use of this technique resulted in a successful viable twin birth.

CASE REPORT

The patient was a 28-year-old when she first sought consultation with us in January 2012, after 1 year and 4 months of marriage. She had married her cousin (III^o consanguinity) and had regular menstrual cycles but infrequent intercourse owing to vaginismus. Her husband's semen parameters were normal and her hormonal evaluation revealed a high day 2 luteinizing hormone (LH) value (>8) in consecutive cycles. A diagnostic hysterolaparoscopy was performed which revealed a posterior wall fibroid (<2 cm)

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patent tubes and normal appearing ovaries. Her uterine cavity was normal with both tubal ostia seen clearly. She underwent a Fenton's repair in the same sitting and was allowed a period of 6 months to conceive using mild ovarian stimulation with clomiphene and timed intercourse.

She returned to us in January 2013 requesting directly for an IVF program. Her hormonal evaluation was done and found to be normal except for a high LH. She was initiated for the long protocol in May 2013, using injection Zoladex 3.6 mg subcutaneously on day 20 (AstraZeneca, London, UK) for downregulation and Gonal-F (Merck Serono, India) with Luvris (Merck Serono, India) for ovarian hyperstimulation. Nineteen oocytes were obtained of which 11 fertilized and 9 embryos were viable for transfer and freezing. Five embryos were frozen, whereas 4 were used for sequential transfer, 2 (6–8 cells) Grade II on day 3, and one expanded blastocyst (III) on day 5.

She conceived in this cycle, but it resulted in a preclinical pregnancy. A menstrual regulation was performed in June 2013. The couple went on a break until August 2013 when they returned for the frozen embryo transfer (FET). She was placed on hormone replacement therapy and had a FET performed on October 7, 2013. Unfortunately, this too ended in a missed miscarriage at 53 days of pregnancy. The chorionic villi obtained by dilatation and curettage were sent for karyotyping, and it revealed trisomy.

The couple then underwent parental karyotyping. The cytogeneticist revealed the karyotype of the husband to be normal male with pericentric inversion of chromosome 9 (46, XY, inv (9) (p11q13)). Although found in 1–1.65% of the population, it has been associated with spermatogenic disturbances in men with infertility by the presence of loops or acentric fragments formed during meiosis. It is often familial and associated with birth defects in the offspring. Her karyotype report was found to be normal.

This time, they were counseled regarding PGS of embryos along with their conventional ART program, and they gave their consent for the same. In March 2014, she was once again initiated with the long protocol, using Injection Zoladex (AstraZeneca, London, UK) for downregulation and Recagon (Organon India Pvt. Ltd., India) and human menopausal gonadotropins (LG Life Sciences, India) for ovarian hyperstimulation. We retrieved 22 oocytes by transvaginal aspiration, of which 17 were fertilized using intracytoplasmic sperm injection. Fifteen embryos cleaved of which we performed biopsy on 10 of them, which were at least 6–8 cells or more in cleavage stage on day 3. Out of 10 biopsied embryos [Figures 1 and 2], 5 were normal embryos, 2 were complex abnormal, 1 was an abnormal embryo (–7, –9), and 2 had no DNA detected in them. Only 2

of the 5 normal embryos developed into hatching blastocysts while the rest were arrested. No blastocysts were generated from the remaining biopsied embryos with a blastocyst formation rate of only 20%. The two normal blastocysts [Figures 3 and 4] were transferred to the patient on day 5.



Figure 1: Embryo biopsy-1



Figure 2: Embryo biopsy-2



Figure 3: Blastocyst



Figure 4: Hatching blastocyst

Her serum beta human chorionic gonadotropin done on the 11th day and 13th day was 427.9 mIU/ml and 791.6 mIU/ml, respectively. An ultrasound on 38th day revealed a twin intrauterine gestational sac with fetal heart pulsations. Since then she progressed well with early fetal screening tests being normal. She had a prophylactic cervical cerclage on June 30, 2014, at 14–15 weeks of pregnancy. Her level II antenatal screening at 22 weeks was normal and her antenatal period was also uneventful. An elective cesarean was performed at 35 weeks on November 19, 2014. She delivered a healthy male and female baby weighing 2.19 kg and 2.26 kg, respectively [Figure 5]. Postnatal evaluation of babies was normal. Both the mother and the babies were healthy upon discharge.

DISCUSSION

We have been performing biopsies since October 2013 for PGS and were very restricted to offering it for patients, selecting only those with repeated failures in ART or those with karyotype variations or abnormalities. However, this year, we extended to all affordable patients who would benefit within the first attempt itself to a greater extent than conventional ART. Since then, we have done 75 cases where 740 embryos were generated. Of these, 512 were biopsied under stringent laboratory conditions. About 124 were found to be normal and 325 were found to be abnormal. The most common errors were complex abnormalities (multiple chromosomal errors were detected in a single embryo). The blastocyst generation rate in the selected normal embryos was 87.5%. The clinical pregnancy rate was 62.5%. The subsequent miscarriage rate and take home baby rates posttransfer of normal blasts were 26.6% and 73.4%, respectively. The karyotyping of chorionic villi from these miscarries yielded normal results. Hence, this validates that the procedure is foolproof in assessing chromosomal normalcy.



Figure 5: Preimplantation genetic screening twins

Human aneuploidy is caused by errors at several distinct stages of oogenesis. More so, it is important to recognize that abnormal embryos do have a blastocyst conversion rate as follows, 40% for trisomy, 21% for polyploidy, and 0% for haploid/monosomic. It is also interesting to note that aneuploidies during the cleavage stage affect the chromosomes 15, 16, 21, and 22 the most and X or Y the least.^[1,2,6]

Trisomic and monosomic (aneuploid) embryos account for at least 10% of pregnancies and for women at advanced age, the incidence may exceed 50%.^[3,4] It becomes imperative to detect these in cases of assisted reproduction when performed for women in older age group who wish to try their own gametes. There is a degree of mosaicism (90% normal and 10% abnormal), one can expect in embryos which hamper the efficacy and accuracy of PGS.^[5]

When a report is normal, it means that all nuclei are normal except for 10% which may be tetraploidy. Uniformly, abnormal means that virtually all nuclei are abnormal. Suboptimal culture condition in early embryo development may “predetermine” mosaic embryos. Qi *et al.* reported that 15.6% of blastocyst and embryos biopsied from women with advanced age group were euploid, whereas 84.4% were aneuploid. PGS procedures do entail 3–5% chances of damage to embryos.^[6]

We are associated with two different laboratories that provide us with array comparative genomic hybridization (CGH) and next-generation sequencing (NGS), respectively. Although the former has a 99.8% concordance with the latter, NGS is fast emerging as an efficient diagnostic tool. Array CGH has enough studies to back its efficacy while NGS although comparable, needs more numbers of cases for validation.

CONCLUSION

The success of PGS is highly dependent on technical competence, embryo culture, and quality and also the presence of mosaicism in preimplantation embryos. In this particular case, the low generation of blastocyst could be due to her antecedent clinical history, but in general as discussed earlier the blastocyst formation rates are much higher in biopsied normal embryos.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, *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.
2. Ly KD, Agarwal A, Nagy ZP. Preimplantation genetic screening: Does it help or hinder IVF treatment and what is the role of the embryo? *J Assist Reprod Genet* 2011;28:833-49.
3. Storchova Z. The Causes and Consequences of Aneuploidy in Eukaryotic Cells. In: *Aneuploidy in Health and Disease*, InTech, European Union. 2012. Available from: <http://cdn.intechopen.com/pdfs-wm/36864.pdf>. [Last accessed on 2015 Dec 15].
4. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: Mechanisms and new insights into an age-old problem. *Nat Rev Genet* 2012;13:493-504.
5. Los FJ, Van Opstal D, van den Berg C. The development of cytogenetically normal, abnormal and mosaic embryos: A theoretical model. *Hum Reprod Update* 2004;10:79-94.
6. Qi ST, Liang LF, Xian YX, Liu JQ, Wang W. Arrested human embryos are more likely to have abnormal chromosomes than developing embryos from women of advanced maternal age. *J Ovarian Res* 2014;7:65.