# Successful pregnancy outcome after *in vitro* fertilisation following Pre-implantation Genetic Diagnosis/Polymerase Chain Reaction screening for single gene disorder (sickle cell anaemia) before embryo transfer: The clinical experience of an *in vitro* fertilisation clinic in Nigeria

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## ABSTRACT

A couple, both carriers of the sickle cell anaemia trait (Genotype HbAS) with an offspring already affected with the genetic disease underwent a Pre-implantation Genetic Diagnosis/ Polymerase Chain Reaction screening of biopsied blastomeres. DNA analysis of single blastomeres was carried out to find out indicated a viable intra-uterine pregnancy with embryos which carried the sickle cell mutation, which resulted in a livebirth (HbAS). PGD/PCR in combination with IVF appears to be the most suitable treatment plan for patients who are at a higher risk of reproducing offspring affected with inheritable genetic diseases.

**Key words:** PGD/PCR, Sickle cell anaemia, IVF, Biopsy, FET

## INTRODUCTION

Sickle cell anaemia is a well-known autosomal recessive disease. Most commonly affects people of African descent. In Nigeria, sickle cell anaemia affects about 1-3% of the Nigerian population. Individuals who have only one copy of the mutation are said to have sickle cell trait.[1,2] These people are usually healthy but can transmit the disease to their children.[3] Sickle cell anaemia is characterised by acute episodes of pain (in the abdomen, chest or joints) and exhibits a range of severity.[4,5] Sickle cell disease is a condition which alters the shape of the red blood cells from round to "sickle" shape, causing them to block small blood vessels and interfere with normal blood flow. Children affected with sickle cell disease experience chronic episodes of pain and an increased susceptibility to potentially life-threatening conditions, including bacterial infections and organ failure. At the present time, there is no cure for sickle cell anaemia. Although there are some altering therapies such as hydroxyurea and hematopoietic cell transplantation for the management of sickle cell disease, but these can have serious side effects and are very risky. They also may not be readily available for all patients.

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The average life expectancy for people with sickle cell anaemia is less than 50 years. The first Deoxyribonucleic acid (DNA) diagnostic procedure for prenatal purposes was reported more than thirty years ago.[6] Subsequently, it was recognised that the mutation itself affected the cleavage site of a restriction enzyme,*DdeI*, that could recognize the DNA sequence of CTNAG (N = A, T, C, or G). While DNA from a normal allele (CTGAG) would be digested by the enzyme, DNA from an affected allele in which A is substituted by T (CTGTG) would not.[7,8] The resulting differences between DNA fragment sizes can then be recognised by electrophoresis, thus forming the basis for diagnosis. With the advent of polymerase chain reaction (PCR), rapid DNA analysis methods have become available, and these techniques are now widely used for prenatal diagnosis.[9-13]

Pre-implantation genetic diagnosis (PGD) is a procedure that has emerged against the backdrop of *in vitro* fertilisation (IVF) technology. In Nigeria, this process of IVF was first introduced by Ashiru *et al*.[14] Following their pioneering work, we were able to advance this technique for patients with infertility,[15,16]and now for patients with advanced maternal age and Sickle cell disorders with the use of PGD. With PGD, the genetic status of an embryo can be determined before transfer into the uterus after IVF, thus almost completely eliminating the risks of bearing a child with the disease[17](PGD is not 100% accurate). The most common form of PGD involves the extraction of one or two cells from the pre-implantation embryo, often around the 8-cell stage. Testing of these extracted cells allow affected embryos to be identified and discarded. Couples at risk for the transmission of genetic diseases can now choose IVF and PGD to avoid the problem of selective abortion that is associated with pre-natal diagnosis during pregnancy.

## MATERIALS AND METHODS

The patient was a 29-year-old woman and spouse with heterozygous genotype (AS). Couple already have a 2-year-old child who is affected with sickle cell anaemia. Prior to ovarian stimulation, hysterosonogram (HSN) was done which revealed normal uterine cavity.

The patients gave their written consent before having PGD/PCR screening. The protocol was also approved by the research and ethics committee of the centre.

Following 3 weeks of oral contraceptive use, the patient was down-regulated using a GnRHanalog (leuprolide). Super ovulation was achieved with the use of recombinant follicle stimulating hormone (FSH). When three or more leading follicles measured >18 mm in diameter, the patient was triggered with *Human Chorionic Gonadotropin* (HCG) 5000 IU. A total of 29 oocytes were recovered 36 hours after the administration of HCG. The surrounding cumulus and corona cells were then removed and the nuclear maturity of the oocytes was assessed under an inverted microscope. Only metaphase II oocytes were injected with morphologically normal motile spermatozoa.

Further culture of injected oocytes was done in 20 µl microdrops of culture medium under lightweight paraffin oil. Fertilisation was confirmed after 16-18 hours by the observation of two distinct pronuclei (2PN). Oocytes with 2PN were assessed on day 2 after injection for embryonic development.

On day 3, a single blastomere was biopsied for embryos at 6-8 cell stage with less than 50% fragmentation [Figures 1 and 2].

Fifteen biopsied cells were sent for genetic screening and analysed using PGD/PCR technique [Figure 3]. Results provided after 48 hours showed that 5 (33%) of the biopsied cells were of genotypes unaffected by sickle cell anemia; of these, three were heterozygous carriers of Hb S (AS) and two were homozygous for HbA (AA)."

## RESULTS

Three unaffected embryos (two heterozygous normal and one homozygous) were transferred on day 5 at blastocyst stage. The remainder of the normal embryos were preserved for future by vitrification. A pregnancy test was done 2 weeks later. The patient tested negative.

Following the failed IVF/PGD cycle, the patient was counselled, reassured and advised to go for a FET. Endometrial lining was prepared for FET using increasing doses of Estradiolvalerate from 2 mg daily up to 8 mg with weekly monitoring of the endometrium. Two vitrified normal embryos (one HbAS and one HbAA) were thawed both at hatched blastocyst stage and transferred [Figure 4].

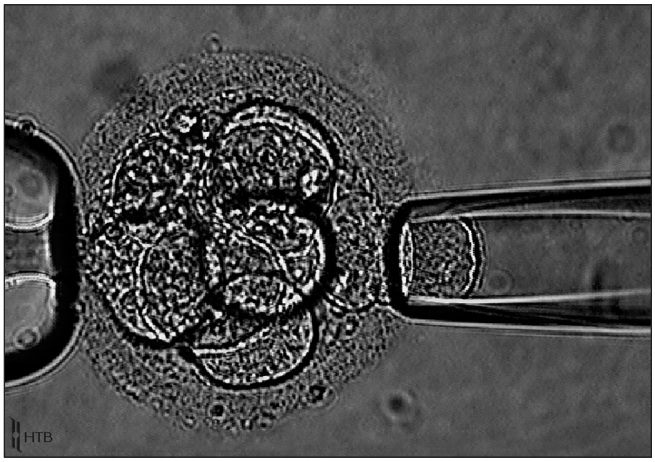


**Figure 1:** Embryo biopsy

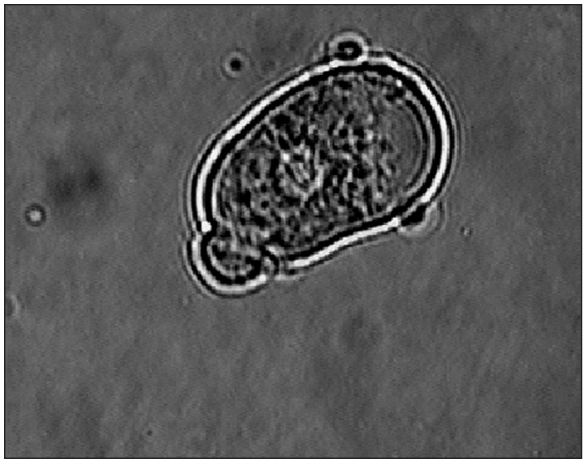

**Figure 2:** Blastomere showing nucleus

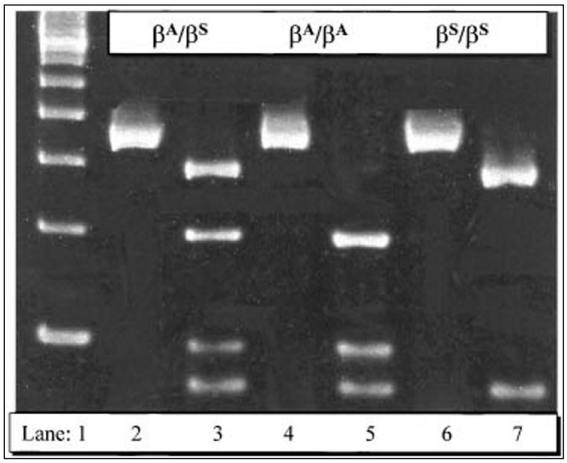


**Figure 3:** Detection of sickle cell disease by polymerase chain reaction (PCR)

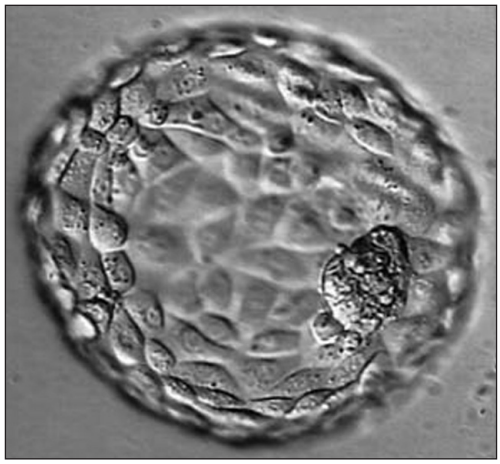

**Figure 4:** A hatched blastocyst

Beta hCG test done 14 days after FET was positive. Clinical pregnancy was confirmed by ultrasound scan at 6 weeks of gestation. The patient delivered a baby boy in May 2012 and the genotype is HbAS.

## DISCUSSION

The advent of IVF as a treatment for infertility has created the opportunity to study the chromosomal constitution of surplus human pre-implantation embryos. Cultured human pre-implantation embryos have been used to develop methods which allow PGD analyses by PCR on biopsied blastomeres from an embryo.[18]

Beta-Thalassaemia and sickle cell anaemia are β-globin chain quantitative and structural disorders that lead to anaemia syndromes. Until recently, the only alternative for couples with a high genetic risk was to undergo Prenatal Diagnosis followed by termination of an affected pregnancy. The PGD of β-Thalassaemia and sickle cell anaemia is an alternative that avoids therapeutic abortion by diagnosing embryos for β-globin defects before implantation into the mother's womb.[19]

On a world-wide scale, PGD for β-thalassaemia and/or sickle cell anaemia has already been applied on single blastomeres[20] and on the first and second polar bodies.[21] The molecular strategies used were DNA amplification followed by genetic diagnosis by denaturing gradient gel electrophoresis analysis,[20] restriction enzyme digestion, the creation of a new restriction enzyme recognition sequence involving the *IVS1 nt 110* mutation[21,22] and the use of fluorescence PCR.[22]

In the present study, PGD was applied clinically for sickle cell anaemia on a fertile carrier couple with previous experiences of therapeutic abortion for affected foetuses, and a sickle cell disease child. Despite the fact that sickle cell anaemia is one of the most common genetic disorders and detailed genetic information is available,[23] unaffected pregnancies following PGD for sickle cell anaemia previously have not been reported. Lack of previous success in this area presumably is due to the length of time and effort required to overcome technical difficulties inherent in these procedures, as well as lack of available research funding.[17] Our results demonstrate that sickle cell anaemia can be detected in single cells by PCR and restriction enzyme analysis and that unaffected pregnancies can be established by the transfer of embryos of known genetic makeup that have undergone biopsy. In total, one PGD cycle with a frozen embryo transfer cycle were carried out.

The goal of PGD is to reduce the risk of having children born with genetic diseases from a *priori* risk of 25% to a value significantly less. However, the risk is not totally reduced to zero. Patients undergoing PGD should be advised that if a pregnancy does occur from this treatment, they should consider undergoing Chorionic Villi Sampling (CVS) or Amniocentesis to confirm these micro-genomic results.

In summary, this is the first unaffected pregnancy and delivery after successful PGD for sickle cell anaemia. Our results demonstrate that PGD for the detection of sickle cell anaemia is a powerful diagnostic tool for carrier couples who desire a healthy child but wish to avoid the difficult decision of whether to abort an affected foetus. The procedure, successfully used in this case, may also be applied to other monogenic disorders and further supports the notion that PGD is destined to be an integral aspect of assisted reproductive technology. Given the current methods and relatively high cost of the procedure, it is unlikely that PGD will totally replace prenatal testing. However, it is conceivable that with further refinements, PGD will certainly become an invaluable and powerful diagnostic modality.

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