CASE REPORT

Live birth following preimplantation genetic testing to prevent sickle cell disease in a low resource setting: A case report

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Abstract

Preimplantation genetic testing for monogenic disorders (PGT-M) can detect sickle cell disease (HbSS) in embryos during In-Vitro Fertilization (IVF), to ensure the birth of unaffected children. The prevalence of haemoglobin S gene (HbS) is high in Nigeria and Sub-Saharan Africa, but access to PGT-M services in the setting is limited. A couple with the sickle cell trait (HbAS) had IVF, following which 12 embryos were biopsied and the corresponding cells analyzed using minisequencing for whole genome amplification and polymerase chain reaction (PCR) to determine each embryo's haemoglobin (Hb) genotype. Two HbAA (normal) embryos were transferred to the mother resulting in the birth of a live HbAA male infant at 38 weeks gestation. The child has remained well after nine months of follow up with HbAA at repeat genotype evaluation. *(Afr J Reprod Health 2020; 24[4]: 218-220).*

Keywords: Preimplantation genetic testing, Sickle cell anaemia, In-Vitro Fertilization (IVF), Nigeria

Résumé

Les tests génétiques préimplantatoires pour les troubles monogéniques (PGT-M) peuvent détecter la drépanocytose (HbSS) dans les embryons pendant la fécondation in vitro (FIV), pour garantir la naissance d'enfants non atteints. La prévalence du gène de l'hémoglobine S (HbS) est élevée au Nigéria et en Afrique subsaharienne, mais l'accès aux services de PGT-M dans le contexte est limité. Un couple avec le trait drépanocytaire (HbAS) a eu une FIV, à la suite de laquelle 12 embryons ont été biopsiés et les cellules correspondantes analysées à l'aide d'un miniséquençage pour l'amplification du génome entier et la réaction en chaîne par polymérase (PCR) pour déterminer le génotype de l'hémoglobine (Hb) de chaque embryon. Deux embryons d'HbAA (normaux) ont été transférés à la mère, entraînant la naissance d'un nourrisson vivant de sexe masculin HbAA à 38 semaines de gestation. L'enfant est resté en bonne santé après neuf mois de suivi avec HbAA lors de la réévaluation du génotype. *(Afr J Reprod Health 2020; 24[4]:218-220).*

Mots-clés: Test génétique préimplantatoire, anémie falciforme, fécondation in vitro (FIV), Nigéria

Introduction

Pre-implantation genetic testing for monogenic diseases (PGT-M) is a useful tool for detecting genetic abnormalities in embryos during in vitro fertilization (IVF) before the embryos are transferred to enable pregnancy[1]. The objective of PGT-M is to avoid transmission of genetic disorders from parents to their children.

Haemoglobin S (HbS) is a common transmissible disorder amongst Africans. The homozygous condition, known as sickle cell anaemia (HbSS), is characterized by chronic haemolytic and thrombo-embolic phenomena, resulting in anaemia, repeated infections, bone pain crises and high mortality[2].A worldwide breakthrough in the prevention of HbSS for a couple at risk of transmitting the HbS genes was achieved in 1991 by Handyside *et al*[3]. Since then, the technology has become available around the world, except for sub-Saharan Africa. In-Vitro Fertilization centers in Nigeria rely on collaboration with genetic laboratories in high income countries for PGT-M. To our knowledge, this is the first report from an indigenous PGT-M laboratory in Nigeria with successful prevention of transmission of sickle cell anaemia

Case Report

A 28-year-old nulliparous (Para 0^{+2}) woman and husband, who were both confirmed to be HbAS,

presented seeking for a child unaffected by HBSS. The woman had no abnormalities on physical examination. The uterus, ovaries and adnexae were normal on ultrasound examination. Semen analysis for the man was normal. They were counseled on available options, either prenatal diagnosis during the early phase of a spontaneous pregnancy by chorionic villus sampling (CVS) or via IVF and PGT-M. They opted for IVF and PGT-M.

In-vitro fertilization

Ovarian stimulation and triggering of ovulation were done using standard institutional protocols. A total of 22 oocytes were retrieved by transvaginal ultrasound guided approach. Fertilization was achieved by intracytoplasmic sperm injection (ICSI), resulting in 18 zygotes, all of which were continued in culture.

Embryo biopsy

On day 3, each of the 14 embryos that attained 8-cell stage were placed in separate microdrops (20µL) of calcium and magnesium-free buffered medium for 3-5 minutes to facilitate dissociation of the blastomeres from each other. The Saturn Laser system was used to create a hole in the zona pellucida, through which a single blastomere was removed from each embryo, using an aspiration pipette and transferred into the corresponding 0.2 µL Polymerase Chain Reaction (PCR) tube, as described by Wang *et al*[4].

Genetic analysis

Each blastomere was lysed and the deoxyribonucleic acid (DNA) subjected to whole genome amplification using SurePlex DNA Amplification System (Illumina Inc., California, USA)[5] according to the manufacturer's instructions, then subjected to two rounds of PCR with specific primers to the Hbb gene. Single nucleotide polymorphism (SNP) detection was by the minisequencing technique. Capillary electrophoresis was performed using Applied Biosystem 3500 Genetic Analyzer (Life Technologies, California, USA) according to manufacturer's instructions. On culture day 5, twelve embryos had reached blastocyst stage of development and were all cryopreserved to avoid ovarian hyperstimulation syndrome. The results of genetic analysis were; 5 HbAA, 3 HbAS, 3 HbSS and one embryo had failed amplification (i.e. no diagnosis).

Embryo transfer, conception and delivery

Two frozen embryos identified to be HbAA were thawed. Both survived and were transferred to the woman. Pregnancy test became positive and a singleton pregnancy was confirmed five weeks after embryo transfer by transvaginal ultrasonography. Pregnancy course was normal until 37 weeks gestation when she developed Pregnancy Induced Hypertension (PIH). She was delivered by elective caesarean section at 38 weeks gestation, with the birth of a male infant weighing 3.6 kg. The infant's Hb genotype was confirmed to be HbAA at birth and at age 9 months. The child has remained well after 9 months of follow up.

Discussion

Collaboration with high income country laboratories in the use of PGT-M for delivery of offspring with normal Hb genotype to prevent sickle cell anaemia in Nigeria has been previously reported[6]. However, the technology has not been locally available within the country. The dearth of genetics laboratories capable of PGT-M technology in low resource countries can be attributed to several barriers. Our experience has been that laboratory equipment and consumables are expensive. Finding local scientists to be sent for training abroad or foreign based experienced geneticists willing to relocate to Nigeria were challenging. Further, maintaining a conducive physical environment for good laboratory practice was difficult. These challenges have been successfully overcome in the present report, making PGT-M service now readily accessible and less expensive for the population.

There is concern about the adverse effects of additional handling and biopsy on the implantation potential of embryos. The outcome in the present report was satisfactory in terms of development, freeze-thaw survival and implantation of biopsied embryos. However, further studies are required to make definite

conclusions. The failure of amplification (no diagnosis) in one of the biopsied cells was likely due to highly degraded DNA, as indicated in another report[7].

Prenatal diagnosis via Chorionic Villus Sampling (CVS) or amniocentesis has been advocated to crosscheck the results of PGT-M, but the procedures carry additional risk of pregnancy loss[8]. The couple in this report declined consent for prenatal diagnosis to avoid this risk. The choice of caesarean section as the mode of delivery was due to PIH, a common pregnancy complication, not related to the PGT-M.

Local availability of PGT-M and successful outcome of delivery of a child with HbAA has important implications for couples with genetic risks in this setting. It creates hope and potential for future prevention and control of genetic conditions, as well as the potential for advancement of research in this field. However, the social, ethical and religious implications of this technology in the setting have not been explored. Discussions in this regard would be helpful to guide scientists and the society on the limits of clinical application of this technology. Although our initial observations suggest that there is good acceptability among couples of diverse religious and social backgrounds, careful ethical regulation is necessary.

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