ABSTRACT
Duchenne muscular dystrophy is the most common muscle disease found in male children. Currently, there is no effective therapy available for Duchenne muscular dystrophy patients. Therefore, it is essential to make a prenatal diagnosis and provide genetic counseling to reduce the birth of such boys. We report a case of preimplantation genetic diagnosis associated with Duchenne muscular dystrophy. The couple E.P.R., 38-year-old, symptomatic patient heterozygous for a 2 to 47 exon deletion mutation in DMD gene and G.T.S., 39-year-old, sought genetic counseling about preimplantation genetic diagnosis process. They have had a 6-year-old son who died due to Duchenne muscular dystrophy complications. The couple underwent four cycles of intracytoplasmic sperm injection (ICSI) and eight embryos biopsies were analyzed by polymerase chain reaction (PCR) for specific mutation analysis, followed by microarray-based comparative genomic hybridisation (array CGH) for aneuploidy analysis. Preimplantation genetic diagnosis revealed that two embryos had inherited the maternal DMD gene mutation, one embryo had a chromosomal alteration and five embryos were normal. One blastocyst was transferred and resulted in successful pregnancy. The other embryos remain vitrified. We concluded that embryo analysis using associated techniques of PCR and array CGH seems to be safe for embryo selection in cases of X-linked disorders, such as Duchenne muscular dystrophy.

Keywords: Muscular dystrophy, Duchenne; Polymerase chain reaction; Comparative genomic hybridization; Prenatal care; Case reports

INTRODUCTION
Duchenne muscular dystrophy (DMD; MIM 310200) is an X-linked recessive disorder that is caused by mutation of the DMD gene (gene ID: 1756) located at Xp21.(1) Duchenne muscular dystrophy is the most common muscle disease in male children, with an incidence of 1:3,500 live born males.(1) The dystrophin gene is the largest identified human genes. It contains 79 exons, at least seven different tissue-specific promoters and two polyadenylation sites. However, dystrophin RNA is differentially processed producing a variety of transcripts encoding a set of protein
isoforms.\(^{(1-3)}\) The protein translated from the larger transcript is an important cytoskeletal protein, which helps the cytoskeleton of each muscle fiber to connect to the underlying basal lamina. Alteration or loss of dystrophin forces excessive amounts of calcium into the cell membrane, resulting in excess water formation in the mitochondria; thus, the affected skeletal muscle will result in dystrophy, mitochondrial dysfunction, and necrosis.\(^{(4)}\)

The majority of identified mutations are deletions, accounting for approximately 60-65\% of DMD. Duplications have been observed in 5-15\% and the remaining cases and they might be caused by small mutations such as microdeletions, microinsertions, point mutations, or splicing mutations.\(^{(1,2,5)}\) Approximately one-third of the DMD patients originate through new mutations, while the rest are inherited through carrier mothers or arise from germ line mosaicism.\(^{(6,7)}\)

Pathologically, DMD is characterized by rapidly progressive degeneration and necrosis of the proximal muscles and calf pseudo-hypertrophy. Most DMD patients show muscle weakness in early childhood, become wheelchair-dependent by around 12 years old, and die for respiratory or cardiac failure in the late adolescence or early 20s. Currently, there is no effective therapy available for DMD patients. Therefore, it is essential to make a prenatal diagnosis and provide genetic counseling to reduce birth of such boys.\(^{(4)}\)

We report a case of preimplantation genetic diagnosis associated with Duchenne muscular dystrophy.

**CASE REPORT**

E.P.R., 38yo, symptomatic patient heterozygous for a 2 to 47 exon deletion mutation in DMD gene and G.T.S., 39yo, sought genetic counseling service at the **Instituto Ideia Fertil, Centro de Reprodução Humana e Genética of Faculdade de Medicina do ABC, Santo André, São Paulo, Brazil** in February 2014 to know about the preimplantation genetic diagnosis (PGD) process. They have had a 6-year-old son who died in 2012 due to DMD complications. The couple underwent four cycles of assisted human reproduction treatment with intracytoplasmic sperm injection (ICSI). Ovarian stimulation was performed by using 200UI of exogenous recombinant follicle stimulating hormone from the second day of the menstrual cycle. When the largest follicle reached 14mm, the antagonist of gonadotropin-releasing hormone (GnRH) was also administered. After, when the largest follicles reached 17mm, human chorionic gonadotropin (hCG) a dose of 5000IU was provided and 36h later the oocyte retrieval was performed. Eight D5/D6 embryos biopsies were analyzed by PCR for specific mutation analysis, followed by array CGH for aneuploidy analysis (screening of 24 chromosomes) in a specialized private laboratory.

The genetic analysis disclosed that two embryos had inherited the maternal DMD mutation, one embryo had a chromosomal alteration [47,XY,del(8)(q24.11-qter),+18] and five embryos (three males and two females) were normal. One blastocyst was transferred and resulted in successful pregnancy. The child was a girl, born after 38 gestational weeks by cesarean section, weighting 2.970kg and measuring 43cm. The other embryos remained vitrified.

**DISCUSSION**

The majority (>90\%) of women with mutations in the DMD gene is asymptomatic. Although, the mutation carrier woman may be affected by DMD as a result of changes in the pattern of X chromosome inactivation, Turner syndrome (45,X) or translocation of X chromosome to an autosome. Women with muscular dystrophy symptoms are often considered as limb-girdle muscular dystrophy patients.\(^{(8)}\) So far, there are few population data on the prevalence of carriers, especially on symptomatic carriers. The knowledge about the condition of carrier pathogenic mutation is important to the genetic counseling, mainly because half of the children of carriers should be affected by this disease and half of the daughters will be the bearer of the pathogenic mutation.

The DMD prevention importance has been widely emphasized, once there is no curative therapy available. For those women at risk, genetic counseling can be offered and also alternatives to prevent transmission of the mutated allele, such as carrier screening tests, human assisted reproduction with PGD or the use of donor eggs and prenatal diagnosis.\(^{(8)}\) The PGD to determine just sex is not enough, especially in our case because the girl could be a carrier of the mutated allele and develop symptoms of DMD, as well as her mother. A male embryo is tested by direct analysis of familial mutation to determine if he is affected. The birth of affected boys can be prevented by terminating the pregnancy in countries where abortion is allowed. In these cases, parents need to face the emotional burden for the possibility to abort a healthy boy, a fact that is the major drawback of prenatal diagnosis of X-linked disorders by sex determination only. By using the PGD, either unaffected female or male embryos can be selected and transferred to the uterus.\(^{(8)}\) It is important to emphasize that perform PGD in cases of monogenic
Preimplantation genetic diagnosis associated to Duchenne muscular dystrophy

In conclusion, the embryo analysis using associated techniques of PCR and array CGH showed to be safe for embryo selection in cases of X-linked disorders, such as Duchenne muscular dystrophy.

REFERENCES


