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Article

Live birth derived from oocyte spindle transfer to prevent mitochondrial disease

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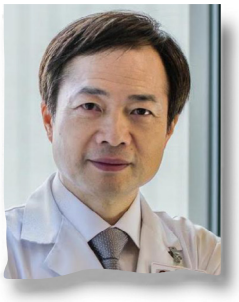

Dr John Zhang completed his medical degree at Zhejiang University School of Medicine in China, and subsequently received his Master's Degree at Birmingham University in the UK. In 1991, Dr Zhang earned his PhD in IVF, and, after researching the biology of mammalian reproduction and human embryology for nearly 10 years he completed his fellowship training in Reproductive Endocrinology and Infertility at New York University's School of Medicine in 2001. Dr. Zhang continues his clinical research in minimal stimulation IVF, non-embryonic stem cell, long-term oocyte cryopreservation, and oocyte reconstruction by nuclear transfer.

KEY MESSAGE

We report a live birth after oocyte spindle transfer to prevent transmission of the mitochondrial disease, Leigh syndrome.

A B S T R A C T

Mutations in mitochondrial DNA (mtDNA) are maternally inherited and can cause fatal or debilitating mitochondrial disorders. The severity of clinical symptoms is often associated with the level of mtDNA mutation load or degree of heteroplasmy. Current clinical options to prevent transmission of mtDNA mutations to offspring are limited. Experimental spindle transfer in metaphase II oocytes, also called mitochondrial replacement therapy, is a novel technology for preventing mtDNA transmission from oocytes to pre-implantation embryos. Here, we report a female carrier of Leigh syndrome (mtDNA mutation 8993T > G), with a long history of multiple undiagnosed pregnancy losses and deaths of offspring as a result of this disease, who underwent IVF after reconstitution of her oocytes by spindle transfer into the cytoplasm of enucleated donor oocytes. A male euploid blastocyst was

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http://dx.doi.org/10.1016/j.rbmo.2017.01.013

obtained from the reconstituted oocytes, which had only a 5.7% mtDNA mutation load. Transfer of the embryo resulted in a pregnancy with delivery of a boy with neonatal mtDNA mutation load of 2.36–9.23% in his tested tissues. The boy is currently healthy at 7 months of age, although long-term follow-up of the child's longitudinal development remains crucial.

Introduction

Mitochondria provide energy for most eukaryotic cells and are assembled with proteins encoded by both nuclear and mitochondrial DNA (mtDNA). At least 1 in 5000 people in the general population has one mutation in mtDNA, which can cause mitochondrial dysfunction and maternally inherited diseases (Gorman et al., 2015). When both wild type (normal) and mutant mitochondrial genomes co-exist, a condition called heteroplasmy, the severity of the symptoms is associated with the level of mtDNA mutation load (Freyer et al., 2012). Leigh syndrome is a devastating childhood disease caused by mitochondrial deficiency. About 20–25% of Leigh syndrome cases are caused by mtDNA mutations (Swalwell et al., 2011). The mtDNA 8993T > G mutation, one of the most common of such mutations, impairs the function of the F0 portion of ATPase causing ATP-synthetic defects. In cells harbouring 8993T > G mutation, mitochondrial ATP synthesis is reduced by 50–70% (Nijtmans et al., 2001), thereby causing failure of the mitochondrial respiratory chain. Patients with Leigh syndrome often develop regression of both mental and motor skills leading to disability and rapid progression to death, often owing to seizures and respiratory failure (Nijtmans et al., 2001; Swalwell et al., 2011). When mtDNA 8993T > G mutation load is less than 30%, the carrier is expected to be asymptomatic. A large cohort study showed that the probability of having severe symptoms, i.e., pathological phenotype, is low until the mutant load (heteroplasmy level) reaches 60–70% for the 8993T > G mutation (White et al., 1999), indicating a high tolerance threshold for mutation load.

Current clinical options to prevent transmission of mtDNA mutations to offspring are limited. A couple could adopt a child, use donor oocytes or use prenatal diagnosis and abort an affected pregnancy. There is no reliable way of pre-selecting embryos with pre-implantation genetic diagnosis for most cases of mtDNA mutation (Mitalipov et al., 2014), particularly for a woman with a high level of heteroplasmy. Nuclear transfer has been proposed as a novel approach to minimize the transmission of mutant mtDNA from a carrier mother to her child at the gamete or zygote level (Craven et al., 2010). Experimental nuclear transfer in both animals and humans has been reported, and our group as well as others has worked on this for over 2 decades (Liu et al., 1999, 2003; Zhang et al., 1999; Tachibana et al., 2009; Tachibana et al., 2013; Zhang and Liu, 2015; Hyslop et al., 2016; Zhang et al., 2016). The tragic consequences of childhood mitochondrial disease, in particular those of Leigh syndrome, prompted the current experimental effort.

Recent studies show that two technicques can be used to carry out nuclear transfer for mitochondrial replacement therapy: metaphase II (MII) spindle transfer and pronuclear transfer (Craven et al., 2010; Tachibana et al., 2009). These techniques, however, have yet to be conducted clinically owing to regulatory constraints in countries in which reproductive techniques are subject to legal and regulatory oversight. Pronuclear transfer leads to discarding of zygotes and may raise religious and ethical concerns in certain populations. This makes the spindle transfer technique preferable to pronuclear transfer. The intent of the treatment described here was to allow a woman, who carries a mitochondrial DNA mutation (Leigh syndrome), and who has a demonstrated history of transmission of the disease to her offspring, to have a male child with minimal pathogenic mitochondria and with no risk of transferring the disease to his offspring.

Materials and methods

Case description

A 36-year-old asymptomatic woman with a history of four pregnancy losses (between 6–16 weeks of gestation, reasons unknown), and two deceased children (at ages 8 months and 6 years) (**Figure 1**) from Leigh syndrome as confirmed by over 95% mutation load, sought assistance to conceive a healthy baby. The patient was asymptomatic and carried the mitochondrial genome mutation 8993T > G in subunit six of the ATPase gene, which is known to cause Leigh syndrome (Holt et al., 1990). Whole mtDNA sequencing analysis using next-generation sequencing revealed 8993T > G heteroplasmy levels of 23.27%, 24.50% and 33.65% in her hair follicles, blood and urine precipitate, respectively (**Supplementary Table S1**) (**Figure 2**). The

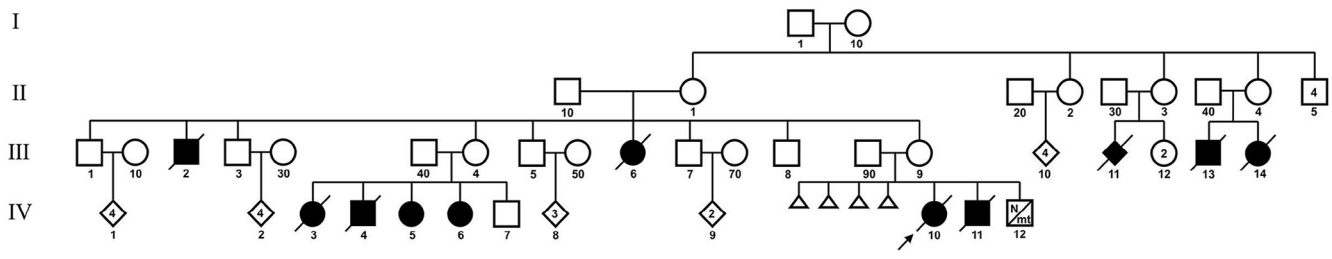

Figure 1 – Pedigree of the family with Leigh Syndrome (black fill indicates clinically affected individuals, blank triangle for miscarriage). The product of the nuclear transfer procedure was indicated by N/mt in order to indicate that the nuclear (N) genome and mitochondrial (mt) genome were from different individuals.