Wednesday, October 19, 2016
11:45 AM

O-267

FIRST LIVE BIRTH USING HUMAN OOCYTES RECONSTITUTED BY SPINDLE NUCLEAR TRANSFER FOR MITOCHONDRIAL DNA MUTATION CAUSING LEIGH SYNDROME. J. Zhang,[1] H. Liu,[1] S. Luo,[2] A. Chavez-Badiola,[3] Z. Liu,[1] m. yang,[1] S. Munne,[4] M. Konstantinidis,[4] D. Wells,[5] T. Huang[6]; [1]New Hope Fertility Center, New York, NY, [2]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, [3]New Hope Fertility Center, Guadalajara, Mexico, [4]Reprogenetics, Livingston, NJ, [5]Reprogenetics, Oxford, United Kingdom, [6]Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

OBJECTIVE: Mutations in mitochondrial (mt) DNA are maternally inherited and can cause fatal or debilitating disorders without effective treatments.[1,2] The severity of clinical symptoms is often associated with the mtDNA mutation load in heteroplasmy.[3] Experimental nuclear transfer in metaphase II (MII) spindle oocytes or in pronuclear (PN) zygotes, also called mitochondrial replacement therapy, has been shown to be a novel technology in minimizing mutated mtDNA transmission from oocytes to pre-implantation embryos.[4,5] Here we report the first live birth of a boy following spindle nuclear transfer (SNT).
DESIGN: Translational research.
MATERIALS AND METHODS: The patient is a 36-year-old woman with 24.5% mtDNA displaying 8993T>G mutation in subunit 6 of the ATPase gene known to cause Leigh syndrome.[6] She had 4 pregnancy losses and 2 deceased children at age 8 months and 6 years from Leigh syndrome as confirmed by >95% mutation load. She was seeking conception of a healthy baby and elected to have SNT over PN transfer for religious reasons. Under IRB-approved protocol with proper consent, the patient's spindle nuclei were isolated and transferred into the perivitelline space of enucleated donor oocytes. The micro-manipulated complexes were then subjected to 1.4 kV/cm DC voltage for cell membrane fusion. The reconstituted oocytes were fertilized by intracytoplasmic sperm injection (ICSI). The developed blastocysts were biopsied for preimplantation genetic screening (PGS) by array comparative genetic hybridization and whole quantitative genomic mtDNA analysis by Next Generation Sequencing.
RESULTS: Five MII oocytes with birefringent spindles were subjected to meiotic SNT. The 5 oocytes were successfully reconstituted and fertilized normally by ICSI. Four out of 5 fertilized oocytes developed into blastocysts. PGS showed that one blastocyst was euploid (46XY), while 3 embryos were aneuploid. The average transmission rate of maternal mtDNA in the biopsied euploid blastocyst was 5.10 ± 1.11% and the heteroplasmy level for 8993T>G was 5.73%. Transfer of the euploid embryo resulted in an uneventful pregnancy with delivery of a healthy boy at 37 weeks of gestation. The average level of transmitted mother's mtDNA in several neonatal tissues including buccal epithelium, hair follicles, circumcised foreskin, urine precipitate, placenta, amnion, umbilical blood, and umbilical cord was less than 1.60 ± 0.92%. The baby is currently 3 months old and doing well.
CONCLUSIONS: Human oocytes reconstituted by SNT are capable of producing a healthy live birth. SNT may provide a novel treatment option in minimizing pathogenic mtDNA transmission from mothers to their babies.

References: 1. Schaefer AM, McFarland R, Blakely EL, et al. Prevalence of mitochondrial DNA disease in adults. Annals of neurology 2008;63:35-9.
2. Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 1988;242:1427-30.
3. Freyer C, Cree LM, Mourier A, et al. Variation in germline mtDNA heteroplasmy is determined prenatally but modified during subsequent transmission. Nature genetics 2012;44:1282-5.
4. Paull D, Emmanuele V, Weiss KA, et al. Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. Nature 2013;493:632-7.
5. Ma H, Folmes CD, Wu J, et al. Metabolic rescue in pluripotent cells from patients with mtDNA disease. Nature 2015;524:234-8.
6. Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. American journal of human genetics 1990;46:428-33.