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Ms. Anna Rajakumar
Senior Policy Officer
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Finsbury Tower
103-105 Bunhill Row
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Dear Ms. Rajkumar,

I write to share my concerns about your plans to consider approving clinical trials of mitochondrial replacement (MR) via spindle transfer (ST) or pronuclear transfer (PT) for clinical indications, including prevention of the transmission of mtDNA diseases and infertility. I served as a voting member at the recent meeting of the U.S. Food and Drug Administration Cellular, Tissue and Gene Therapies Advisory Committee, which addressed this issue. This topic holds special interest to me because I am an academic reproductive medicine specialist, and my laboratory was the first to develop and report the spindle transfer technique in mammals (Nature Biotechnology, 2000;18:223), the non-invasive self-referencing oxygen sensor to assess mitochondrial function in individual oocytes and embryos (Zygote 2000;8:15) and the presence of mtDNA mutations in human eggs (Fert & Steril, 1995;64:577). The application of the ST and PN techniques to macaques and humans represent intriguing advances of earlier work, but displays of technical virtuosity should not blind us to potential hazards of these technologies nor to overestimate the scope of their applicability. Our own group moved away from this research because PGD provides a relatively safe alternative to MR for the majority of patients, and because vexing concerns linger about the safety of MR, including the safety of reagents employed during MR, carryover of mutant mtDNA during MT and disruption of interactions between mtDNA and nuclear DNA (nDNA).

Preimplantation Embryo Diagnosis (PGD) Provides a Safe and Effective Alternative to MR: PGD of oocytes and embryos from women afflicted by MELAS, the most likely candidates for MR, can identify oocytes bearing only low levels of heteroplasmy, thus providing a less heroic but safer and more practical alternative to MR. Women homoplasmic for MELAS, who would not benefit from PGD because they would be unlikely to yield unaffected oocytes, should not become pregnant because of the detrimental impact of pregnancy on their own metabolism. Nor is MR likely to benefit infertile women. The fact that even high burdens of mtDNA mutations in oocytes do not disrupt their developmental potential undercuts the argument that mtDNA mutations contribute to age related oocyte dysfunction. Indeed, we discovered that the reproductive aging phenotype in mice segregates with the nucleus, not the cytoplasm (Biol Reprod 2004;71:1724).

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Safety of Reagents Employed by ST and PT: The original method for ST (Nature Biotechnology, 2000; 18:223) employed electrofusion, but recent studies in macques and humans have taken to Sendai virus. The safety of exposure to this inactivated virus during early human development should be established before trials in humans are approved.

mtDNA Carryover: *Carryover of mtDNA is inevitable during all MR. Both the ST and PT* techniques minimize carryover, but mutant mtDNA can preferentially replicate as part of the cell's compensatory response to underproduction of ATP by some mitochondria. Moreover, the perinuclear location of the mtDNA carried over during MR can allow their preferential replication by nuclear encoded factors regulating replication. Finally, the existence of mutant mtDNA species in the woman's genome demonstrates that the mutant mtDNA carried over with the karyoplast and the nDNA have evolved a favorable, semi symbiotic relationship, which may not be enjoyed by the donor mtDNA. Studies of the dynamics of mutant mtDNA species after nuclear transfer and ST in iPS cells produced from patients with mtDNA diseases could help establish the risk of mutant mtDNA carryover after MR.

mtDNA/nDNA Incompatability: Placental and growth abnormalities, such as the large calf offspring in cattle and sheep produced by nuclear transfer persist even in nuclear transfer embryos grown in vivo, so these are not merely culture media effects. ST and PT transfer chromosomes which are better synchronized by developmental stage, but the possibility that these complications arise from disruption of mitochondrial and nuclear DNA (nDNA) interactions cannot be ruled out.

mtDNA developed a symbiotic relationship with our eukaryotic ancestors over three billion years ago. Mitochondrial replication, transcription, translation and respiration depend on interactions between RNAs and proteins encoded by both the mitochondrial and nuclear genomes. This relationship largely enjoys a mutually beneficial détente, but extensive evidence in model organisms, including yeast, fly, mouse and primates, demonstrates the precarious nature of mtDNA/nDNA interactions. Epistatic interactions between mtDNA and nDNA contribute to phenotypic and fitness variations within species. Milk production in cattle, male and female fertility in flies and behavior in mice are affected by introducing even seemingly neutral variations in mtDNA into different nDNA backgrounds.

The *most striking evidence for co evolution of mtDNA and nDNA and for mitochondrial-nuclear* incompatibility comes from species with especially high rates of mitochondrial evolution, such as primates, suggesting special caution when applying MR to humans. Investigators purporting the safety of ST and PT hold out healthy offspring from racial admixtures as examples of healthy heteroplasmy. This example has limited applicability to ST and PN because MR methods bypass the extensive selective pressures on mtDNA/nDNA incompatability exerted during early development. Moreover, racial and ethnic admixture does not model the mixture of primarily benign and disease causing mtDNA mutations resulting from ST and PT. The metabolism of oocytes, eggs and embryos during the first days of development exhibits the Warburg effect. It does not rely on OXPHOS until blastocyst stage, which presumably explains why patients with severe mtDNA

mutations, such as MELAS, remain fertile. MR produces novel combinations of host and donor mtDNA, and bypasses the naturally occurring vetting process. This could prove especially detrimental when new mixtures of mtDNAs include a mutant mtDNA specifies. Some have proposed that the maternal mode of transmission of mtDNA provides a relatively safe paradigm for initial studies of MR in humans by producing only male offspring. Unfortunately, however, male sterility has been a recurrent phenotype of mtDNA/nDNA mismatches, presumably because the male reproductive system does not benefit from the mtDNA bottleneck acting during early oogenesis. Detrimental mtDNA/nDNA incompatibilities could have lethal effects on their own, or may drift to fixation and lead to incompatibilities later in life when the organism is stressed and/or accumulates new mtDNA mutations. Thus, studies on the safety of MR in macaques should establish safety not only in well controlled laboratory settings, but also in the more clinically relevant human condition, including the "fish and chips stress test" of exposing the animals to stressful diets, and they should extend to later in life, to establish its long term safety.

Thank you for considering these points.

Sincerely yours,

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