NEWS AND COMMENTARY

Mitochondrial DNA therapy

The battle to prevent the transmission of mitochondrial DNA disease: Is karyoplast transfer the answer?

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Investigators have been perplexed for a number of years over how to prevent the transmission of mutated or deleted copies of the mitochondrial genome (mtDNA) from one generation to the next. These rearrangements of mtDNA give rise to the onset of a large number of severely debilitating and even lethal mitochondrial diseases that occur as a result of decreased ATP output from the electron transfer chain (ETC). The perplexity revolves around the mitochondrial genome not following Mendelian patterns of inheritance; instead, it is transmitted from the population present in the fertilized oocyte once the sperm's mtDNA has been eliminated.¹ At or around gastrulation, mtDNA is then randomly segregated to precursor cells that will subsequently differentiate into specialized cells contributing to the offspring's tissues, organs and gametes.²

The dilemma for women who are carriers of mtDNA rearrangements is several-fold. The mutant load within individual oocytes cannot be predicted without using an invasive sampling procedure beforehand, owing to random segregation having occurred in individual oocyte precursor cells, the primordial germ cells. Consequently, there can be large variability in mutant loading between oocytes from the same woman.³ Again, with respect to segregation, if the mutant loading were sufficiently high in the oocyte, it would not be possible to determine whether the load would segregate to cells that would be affected by loss of ATP production, such as neurons, retinal and muscle cells. Preimplantation genetic diagnosis (PGD) offers the potential to assess the mtDNA

content in one blastomere and therefore predict whether the others would surpass the threshold of tolerability for the persistence of mutant mtDNA molecules.⁴ Although this offers hope for the present generation, that their offspring will be largely free of mtDNA disease, any subsequent female offspring will be potential carriers as the mutant molecules could segregate at sufficiently high levels to their primordial germ cells. However, the embryos from some of these women would fall into a grey area and potentially pose a risk if they were transferred, implying that amniocentesis might also be required with a view to potential termination.

The recent advances in assisted reproductive technologies have provided opportunities to determine whether specific manipulations can be used to generate offspring free of their mother's transmissible mutant mtDNA. Spindle transfer (ST), essentially a modified cloning technique, transfers the meiotic spindle and attached chromosomes from one mature oocyte to another in order to select for a cytoplasm or mtDNA background (see Figure 1). This has previously been successfully used to generate both cattle and mice following subsequent fertilization.^{5–8} However, more recently, it has been used to generate live monkeys (*Macaca mulatta*) following sperm injection.⁹ Although the original studies did not attempt to determine the mtDNA content in their respective offspring, the monkey studies suggested that the resultant offspring were homoplasmic for recipient oocyte mtDNA. Although such an outcome appears to offer hope for the future, we strongly suggest that considerable

caution should be taken when interpreting these results, especially when considering the appropriateness of the assay sensitivities and controls that were used to support the conclusions. Of particular concern is the lack of evidence to support the conclusion that no mtDNA accompanied the spindle as it was transferred from one oocyte to another.

Our arguments favouring considerable caution are based on well-documented evidence. Similar techniques, such as germinal vesicle transfer (GVT) and pronuclear transfer (PNT), have also been used to prevent the transmission of mtDNA from one generation to the next. However, they have largely been hindered by the transfer of a small population of mtDNA accompanying the karyoplast as it is transferred from the carrier's to the recipient's cytoplasm. This can result in the accompanying mtDNA being fixed at variable levels ranging from 0% to 69%, following, for example, PNT.¹⁰ However, many of the GVT and PNT studies have been performed on mouse models and, as the period of gestation is much shorter than in humans, it is difficult to predict whether a greater accumulation of mutant mtDNA would arise in the human fetus at a period of time when mtDNA accumulates its greatest mass.¹¹ These outcomes are similar to those observed with somatic cell nuclear transfer, in which mtDNA accompanying the donor cell can constitute up to 59% of the animals' total mtDNA content (reviewed in St John *et al.*¹²). Other studies have demonstrated that following nuclear transfer the mtDNA-specific nuclear-encoded replication factors are not silenced, as is the case for fertilized oocytes.^{13,14} Consequently, during the first few embryonic divisions, karyoplast mtDNA will be in the close vicinity of the replication factors, thus ensuring its preferential replication. Even levels as low as 0.002% of the original karyoplast mtDNA contribution to the zygote can be detected at the hatched blastocyst stage, indicating that these molecules are present just before gastrulation, during which mtDNA will be randomly segregated and replicated, and

Figure 1 Spindle transfer. Once the metaphase II spindle has been removed from the mature oocyte of a carrier of mtDNA disease, it is transferred into an enucleated oocyte from an oocyte donated by a female who does not carry mtDNA molecules associated with mtDNA disease (recipient). The reconstructed oocyte can then be fertilized through either *in vitro* fertilization or intracytoplasmic transfer (ICSI), cultured *in vitro* and transferred to the uterus of the 'chromosomal' mother. The processes of somatic cell nuclear transfer, pronuclear transfer and germinal vesicle transfer are essentially similar, as they transfer a karyoplast that is most likely accompanied by mitochondria housing mtDNA.

would continue to be randomly segregated and replicated at each cell division.¹⁵

Furthermore, oocyte mtDNA transferred from one oocyte to another can be transmitted to the subsequent offspring. This was certainly the case following double nuclear transfer, resulting in the generation of live cloned pigs¹⁶ in which mtDNA accompanying the transferred pronuclei was detected.¹⁷ This has also been evident from bovine blastomere nuclear transfer.^{18,19} Furthermore, from cattle hand-made cloning, the fusion of a somatic cell to single or multiple enucleated oocytes resulted in the offspring being either homoplasmic for one population of mtDNA or heteroplasmic, which is again a random outcome. In addition, the use of donor blastomeres to generate the only live cloned non-human primates (*M. mulatta*), derived from a fertilized oocyte as nuclear donors, resulted in transmission of donor blastomere mtDNA.²⁰ This comprised both the mtDNA from the sperm and the oocyte used to generate the embryo, indicating that even minimal amounts of mtDNA introduced into the recipient oocyte are at risk of being transmitted. In

the case of sperm mtDNA, this would represent 0.0001% of the total zygote mtDNA population. In one recorded case of sperm mtDNA transmission to a patient, the patient presented with 90% of the mtDNA content in his muscle tissue being from his father's sperm, which along with a novel 2-bp deletion mediated the phenotypic onset of his myopathy.²¹ From the limited availability of tissue, it appears that, in this individual, only muscle was affected. Consequently, in experimental models designed to demonstrate that homoplasmy can be achieved following karyoplast transfer, multiple tissue types should be analysed in order to be absolutely certain that no heteroplasmy results from the process.

Finally, as the technique of ST transfers the oocyte chromatin from one cytoplasmic environment to another, which may or may not be equivalent, it may therefore have unknown effects on subsequent epigenetic programming during embryo and fetal development. Although previously published ST studies have reported successful development in both cattle and mice,⁵⁻⁸ it should be noted that ST into different cytoplasmic environ-

ments may affect the frequency of development.⁶ Interestingly, and again related to mtDNA, handmade cloning demonstrates that the predominant population of mtDNA in live, healthy offspring was always slightly more genetically diverse than that of the donor cell and the populations from the other cytoplasmic partners, suggesting that careful selection of compatible nucleo-mtDNA partners is required to ensure sufficient levels of ATP will be generated through the ETC,²² the only cellular metabolic process encoded by both the chromosomal and mtDNA genomes. Furthermore, studies on ST, GVT and PNT need to clearly address any epigenetic modifications that may have occurred post-parturition and longitudinally. The consequences of using these methods in an attempt to cure mitochondrial disease may result in epigenetic disorders, as have been reported following other human assisted reproductive treatments.²³⁻²⁶ The question that therefore begs to be answered is whether, once we have solved the issue of mtDNA carry-over, the offspring would fall victims of epigenetic disease—surely, a high price to pay!

Conflict of interest

Drs St John and Campbell are inventors of a patent: Cloning methods and other methods of producing cells; Number WO03/057863. ■

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