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Undergraduate Entry

WINNING ESSAY

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The Current State of Mitochondrial Replacement Therapy

Advances in science often court controversy, however those involving the experimentation and genetic manipulation of human embryos attract more than most. Mitochondrial replacement therapy (MRT) has faced this barrier, with the press touting the phrase “three parent babies” and declaring the inevitability of “designer” children [1]. Despite this, in 2015 the UK became the first country to legally approve the use of MRT in humans [2]. This was followed by the news that a team of US scientists operating in Mexico had already succeeded, resulting in a baby born in 2016 [3]. There are still some questions regarding the clinical efficacy and safety of this technique. These must be addressed, but weighed against the possibility to help families devastated by mitochondrial disease - who have no other treatment options - MRT seems undoubtedly necessary and desirable [4].

MRT Techniques:

Mitochondrial disease is maternally inherited as embryos receive all of their mitochondria from the oocyte. Post-implantation genetic diagnosis can be used to select embryos with the least mutant mitochondrial DNA (mtDNA), however it is not always possible to sufficiently reduce this number. Using donor mitochondria ensures embryos can be created with minimal chance of developing mitochondrial disease or being carriers for subsequent generations. Originally two techniques were developed to achieve this - pronuclear transfer (PNT) and maternal spindle transfer (MST) [5]. More recently pre-pronuclear transfer (PPNT) and polar body transfer (PBT) have been developed [6,7].

Initial feasibility studies into human PNT were carried out using abnormally fertilised embryos [8]. These studies found that maternal mitochondrial DNA (mtDNA) could be reduced to <2% and the resulting embryos could develop to blastocysts. These promising results justified using normal embryos to develop a method for clinical use. The initial technique was not well tolerated by normal embryos, and so was adapted to transfer the pronuclei earlier (shortly after meiosis instead of shortly before the first cell division) [9]. Carryover of mtDNA was reduced to <2% in the majority of blastocysts, and these embryos developed to blastocysts as successfully as controls with no notable differences in gene expression [9]. The success of this study led to the UK’s Human Fertilisation and Embryology Authority (HFEA) to allow the limited clinical use of this therapy- a world-first decision [10].

Before human trials were carried out, MST was performed using macaque embryos, resulting in four healthy animals [11]. This was significant in creating a preclinical method, and demonstrating the safety of this technique by showing healthy postnatal development in primates. Further research by Tachibana et al. using human oocytes produced embryos with successful blastocyst development, normal karyotypes, and <1% maternal mtDNA [12]. A significant number of oocytes however showed abnormal fertilisation, due to perturbation of the maternal spindle and failure to create the second polar body [12]. This low fertilisation rate appears to have been rectified in more recent MST experiments, contradicting the HFEA report that MST may not be as efficient as PNT [13,14].

PBT and PPNT were developed as methods which could isolate the maternal karyoplast without the need for cytoskeleton disrupters as in PNT and MST, the safety of which has not been thoroughly investigated [6]. The technical feasibility of PPNT has been demonstrated, however studies in animal models are needed before use in human embryos [6]. Transfer of both the first (PB1T) and second (PB2T) polar bodies has been carried out in mice to successfully generate offspring. PB1T very successfully limits mtDNA carryover, with no detectable levels in mice after two generations. PB2T showed slightly higher carryover however this was lower than for PNT [15]. PB1T has also been carried out using human oocytes to successfully generate blastocysts. Notably there was only 0.26% average mtDNA carryover, as the first PB is smaller than the spindle or pronucleus and has fewer attached mitochondria. The PB membrane also protects the germline, shown in the high success and genetic integrity of derived embryonic stem (ES) cells [7]. Whilst studies are needed to test PB2T in human embryos, both PBT and PPNT appear to be very promising methods of MRT. The success of these techniques and the omission of cytoskeleton disruptors shows they are worth developing for clinical use.

Questioning Nuclear-Mitochondrial "Incompatibility":

Evolutionary biologists have questioned the safety of MRT by proposing incompatibilities exist between nuclear DNA (nDNA) and certain mtDNA haplotypes [16]. Evidence for this is drawn from mouse studies which claim nuclear-mitochondrial mismatches adversely affect fitness [17,18]. However, these studies used highly inbred populations, raising questions as to their significance with regards to highly outbred and heterogeneous human populations. Other studies breeding genetically distant mice have obtained results showing no nuclear-mitochondrial incompatibilities [19]. Furthermore, the four macaques born using MST show no defects, and were bred from individuals with a high degree of genetic difference [12]. One

should also consider children born to interracial couples, where if nuclear-mitochondrial mismatch was deleterious this cohort would show increased mitochondrial disease, however there is no evidence of this [4].

The theory supporting nuclear-mitochondrial incompatibility is that as mitochondria are maternally inherited mtDNA only co-evolves with female nDNA, leading to male-specific deleterious mutations [20]. Leber hereditary optic neuropathy (LHON), a mitochondrial disease affecting mainly males has been argued as an example of this. No nuclear gene has been identified however that could account for nuclear-mitochondrial incompatibility in males causing LHON, and as some females are also affected this further invalidates the theory [4]. Research has also suggested that it is simply a lack of oestrogens in males which is creating this gender discrepancy [21]. It was further argued that male infertility could be a consequence of nuclear-mitochondrial incompatibility, as sperm motility is highly reliant on mitochondrial function [20]. A UK study however showed no influence of mitochondrial haplotype on sperm motility [22].

Overall there is a lack of evidence to support nuclear-mitochondrial mismatch being deleterious in either sex, however there are still calls for haplotype matching in MRT to avoid mismatches [23]. A recent study investigated the natural occurrence of nuclear-mitochondrial mismatches using data from the 1000 genomes project [16]. This found evidence of individuals with highly related nDNA having extremely divergent mtDNA, and was seen not only in highly admixed populations, but also low admixed populations [16]. These results convincingly show that divergent nDNA and mtDNA haplotypes are naturally occurring in healthy individuals, discrediting the theory of nuclear-mitochondrial mismatch. Clearly previous studies on inbred mice showing deleterious consequences from nuclear-mitochondrial incompatibility do not accurately reflect human populations, indicating the need for caution when using such model organisms to investigate human evolutionary genetics. Nuclear-mitochondrial mismatches are therefore not likely to threaten the safety of MRT, and are not cause for haplotype matching.

Reversion to Maternal Haplotype:

Currently the most significant argument against MRT is the issue of maternal mtDNA carryover. MST can create embryos with >99% donor mtDNA, which in an individual would eradicate any chance of developing mitochondrial disease. However, some ES cell lines derived from these embryos showed reversion from the donor to maternal mtDNA haplotype [9,13]. Kang et al. found certain mtDNA polymorphisms which allow faster mtDNA

replication, or confer a proliferative advantage to cells [13]. In two ES cell lines that reverted to maternal mtDNA a polymorphism was identified in the conserved sequence box II (CSBII) of the D-loop region. This polymorphism affects the efficiency of mitochondrial transcription termination and replication primer genesis, thus affecting the efficiency of mtDNA replication [24]. In these ES cell lines the maternal G6AG8 haplotype conferred a fourfold increase in replication primer synthesis compared to the donor G5AG8 haplotype. Maternal mtDNA would therefore be more efficiently replicated, creating bias towards this haplotype [13]. For two other reversion cell lines specific polymorphisms were not identified, however the D-loop region was again suspected, and in these cases the maternal mtDNA provided a proliferative advantage to cells. Cells with maternally biased heteroplasmy therefore outcompete other cells, causing genetic drift towards maternal mtDNA. No changes in mitochondrial enzyme activity were detected, prompting questions as to the molecular basis of this advantage [13].

The issue arising from these findings is clear; embryos with minimal maternal mtDNA could undergo reversion to the disease-causing haplotype during development. As it was also found that reversion can occur in differentiated cells this could arise after birth resulting in children with disease [13]. Further research is needed to understand the genetic factors underlying the replicative advantages of these mtDNA haplotypes. Haplotype matching focussing on the D-loop region seems prudent to avoid reversion to maternal mtDNA. This is a blow to those pioneering MRT, as the therapy can currently only claim to reduce the risk of transmission, rather than completely prevent mitochondrial disease [5].

Human Births from MRT:

The first human birth resulting from MRT was described in a short report by Zhang et al. in 2016 [3]. The illegality of MRT in the USA was circumvented by carrying out the procedure in Mexico. This news was received with trepidation from the scientific community and it took six months for a comprehensive article [25] to be published. Many concerns have been raised over the methodology and results, a full critique of which was published alongside the article [26]. Subsequently another child has been born using MRT to treat infertility [27]. Births resulting from MRT are likely to become increasingly common, and it is paramount that scientists carrying out this therapy use all research available to determine the most safe and efficacious approach.

Conclusion:

MRT has faced much controversy during its development from a laboratory technique to a clinically approved therapy [5]. The claim that nuclear-mitochondrial DNA mismatch could be deleterious for children born using MRT has been widely dissected and discredited [4]. The safety of MRT seems steadfast, however problems with maternal mtDNA carryover and reversion raise questions over efficacy. Until this problem can be solved MRT cannot claim to irrevocably prevent mitochondrial disease. It will however significantly reduce the risk of women having affected children, or those children being carriers for future generations [5]. Newer techniques such as PBT and PPNT may prove able to eradicate mtDNA carryover and should be clinically developed [7]. The implementation of this technique should begin with controlled clinical trials, and solo efforts such as that by Zhang et al. will not necessarily help the scientific community to develop this technique. There is a plethora of evidence that MRT is safe and can help prevent mitochondrial disease, but ultimately until more countries pass the legislation needed to approve this therapy few families will benefit.

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