

Editorial

First birth following spindle transfer for mitochondrial replacement therapy: hope and trepidation



In this issue, [Zhang et al. \[2017\]](#) report the birth of a healthy boy after mitochondrial replacement therapy (MRT) by spindle transfer to prevent transmission of mitochondrial disease from mother to child. The case was first publicized in the lay press ([Hamzelou, 2016](#); see also editorial by [Johnson, 2016](#)) and then presented during the 2016 Annual Meeting of the American Society for Assisted Reproduction (ASRM) in October 2016 ([Zhang et al., 2016a](#)). It followed an earlier report of an unsuccessful attempt at MRT by pronuclear transfer by the same group ([Zhang et al., 2016b](#)). This world-first birth represents an achievement and a steppingstone, and it has played a role in encouraging the Human Fertilization and Embryology Authority (HFEA) in the UK to issue a final recommendation that the technique 'be approved for cautious use in specific circumstances'. (<http://www.hfea.gov.uk/10559.html>)

We, the editors, were unanimous in deciding that this paper should be published in *RBMO*, based on our conviction that the scientific community must be informed of the details of the work in full in order to evaluate it critically and discuss it openly. We decided this despite the fact that the work has weaknesses and limitations in a number of areas. Moreover, although we were able to encourage the authors to include more details of their work in the submission, some uncertainties concerning methodologies and results still remain. Here we outline our concerns regarding the approach and the treatment process described by Zhang and colleagues.

IRB approval, informed consent, and follow-up plans

The patient's treatment was carried out at two locations. While the ovarian stimulation cycles and oocyte manipulations were carried out at a private fertility clinic in New York, the vitrified embryo was then shipped to Mexico to be warmed and transferred to the patient at an affiliate clinic in Guadalajara. The authors received approval from the Internal Review Board (IRB) of the Mexican clinic for transfer of a 'euploid embryo resulting from cell reconstruction'. The Board also approved a general protocol that included spindle transfer, oocyte reconstitution, intra-cytoplasmic sperm injection (ICSI), and preimplantation genetic screening (PGS). However, no IRB approval for these procedures was applied for in the clinic in New York, contrary to the recommendations of the American

Medical Association regarding international research ([AMA, 2010](#)). Moreover, the accompanying consent form discussed MRT only in a superficial manner, stating that transfer of the meiotic spindle is intended for correction of cytoplasmic disorders, one objective of which 'can be extended to cancel or reduce the risk of mitochondrial disease'. The consent form appropriately stated that 'cell stage reconstruction' through cytoplasmic replacement is considered an 'experimental procedure' but while alternative options to this treatment were listed, risks specific to mitochondrial replacement were not included. The authors explain that the patient received extensive counselling over the course of several years, but the final consent form does not record this.

Although the egg donor reportedly signed a standard egg donor consent form, a copy of this form received by *RBMO* shows that the use of the donated eggs specifically for spindle transfer (for MRT) is not mentioned.

The authors detail their comprehensive plans for follow up of the child, including physical examination every 3 months during the first year and every 6 months during the second year, then annually until age 18, followed by fertility and other assessments after age 18. They state that the patient has agreed to these plans. Indeed, the patient did provide informed consent to testing of neonatal tissues, as well as to follow up 'physical examinations' of the child, but the nature of these examinations or their frequency was not included in the consent form or in the IRB protocol. On the other hand, we understand that signing a consent form does not bind a patient to agree subsequently to often-intrusive medical follow-up studies ([Johnson, 2016](#)). Indeed, informed consent provides for the right of any subject to 'discontinue participation [in a study] at any time without penalty or loss of benefits to which the subject is otherwise entitled' ([US Department of Health and Human Services, 2010](#)) unless early withdrawal threatens adverse effects, in which case these should be described in the consent form. However, we noted that potential adverse effects of MRT were not described in the consent form signed by the patient. Moreover, examination of the patient's child after age 18 would require the child's explicit consent, which may or may not be provided.

Thus, although the requirements for IRB review and approval of this experimental procedure, as well as informed consent, were generally met, the shortcomings of the process may be considered significant and should be fully addressed in future cases.

Patient screening and selection

The 36-year-old patient is a carrier of mitochondrial DNA mutation 8993 T>G, which causes Leigh syndrome. She was appropriately tested and was found to be heteroplasmic with 23%, 24% and 33% pathogenic mtDNA in her hair follicles, blood and urine, respectively. The family pedigree was also examined; the patient has two deceased affected siblings, and one sibling with 4 affected children. Although she is asymptomatic, she has suffered the devastating death of two of her children at ages 8 months and 6 years from Leigh syndrome. She has also experienced a number of miscarriages, though these were not diagnosed as being due to Leigh syndrome. Thus the authors met the requirement for careful patient screening and selection prior to initiating treatment, though no testing was done in the extended family.

Preclinical work and treatment details

The authors report that the patient underwent two minimal ovarian stimulation cycles. Mature oocytes from the first cycle were vitrified until the second cycle was attempted, at which time they were warmed and, along with the fresh oocytes, were available for use in MRT. Donor oocytes were also vitrified and warmed. The impact of vitrification and warming of patient and donor oocytes on the outcome of spindle transfer has not been fully investigated. In the study of [Kang et al. \(2016\)](#), 4 of 6 reconstructed oocytes using vitrified cytoplasm (donor oocytes) with fresh spindles (patient oocytes) fertilized normally and 1 of 4 formed a normal blastocyst. By comparison, 2 of 6 reconstructed oocytes using vitrified spindles (patient oocytes) with fresh cytoplasm (donor oocytes) fertilized normally and one of 2 formed a normal blastocyst. The small sample sizes do not permit a clear interpretation, although the results perhaps suggest that vitrification and warming have minimal effect on the procedure. In the case reported by [Zhang et al. \(2017\)](#), no embryos resulted from vitrified patient oocytes and the transferred embryo was from fresh spindle transfer into vitrified-warmed cytoplasm.

Of the 29 oocytes retrieved from the patient during two cycles, only 9 were found to be mature and even fewer showed birefringent spindles when examined by polarized light microscopy. A total of 15 oocytes were described as 'degenerate'. The reason for such a high incidence of degeneration is unknown. [Zhang et al. \(2017\)](#) do not discuss oocyte quality in any depth, but a recent study by [Kang et al. \(2016\)](#) noted lower peak oestradiol concentration and fewer oocytes from carriers of mitochondrial disease compared with donors. [Kang et al. \(2016\)](#) point to collection of only atretic eggs in one Leigh syndrome carrier patient and although they cite elevated progesterone prior to HCG administration to explain this outcome, it is conceivable that oogenesis and follicular development in women carrying pathogenic mitochondrial DNA is impaired. Based on the reports from [Kang et al. \(2016\)](#) and [Zhang et al. \(2017\)](#) on Leigh syndrome patients, it could also be postulated that completion of maturation in normal oocytes is under the control of ooplasmic mitochondria, whereas the early growth of immature oocytes is not. This is both interesting and surprising, and requires further research. It also shows that simply discarding atretic oocytes, without determining mtDNA mutation rates in the cytoplasm, is a waste of potentially useful material.

According to the authors, this is the first and only clinical MRT case performed by the group, and the approach taken was based on experience gained and experimental data obtained over the last two decades by the authors and others. A number of these publications are cited. Included in their pre-clinical studies are limited data that suggest a 'modified electrofusion' technique does not lead to increased aneuploidy levels following human pronuclear transfer (PNT) ([Liu et al., 2015](#)). But it is not clear how modifications such as opening the zona pellucida using laser ablation (rather than zona dissection) and spindle transfer at 37°C (rather than at room temperature) can address concerns with electrofusion, as the authors do not present any experimental data to support their claim.

Indeed, in the present case, 3 of the 4 biopsied and tested blastocysts were found to be aneuploid by array CGH. Aneuploidy rate is age dependent and can vary from cycle to cycle during assisted reproduction; thus it is difficult to know whether or not electrofusion contributed to aneuploidy in this case. However, a number of authors have suggested that using an electrical pulse is not the preferred method for transfer of spindles, noting that the process may lead to premature activation of the human oocyte and later, abnormal pronuclear formation ([Tachibana et al., 2013](#)). It has been argued that this problem would be avoided if fusion were facilitated via the use of Sendai virus ([Tachibana et al., 2009](#)) or if manipulations were performed in a Ca^{2+} -free medium ([Tachibana et al., 2013](#)). On the other hand, [Paull et al. \(2013\)](#) suggested that premature activation and aneuploidy could be avoided by shortening the interval between ovulation trigger and oocyte retrieval and by short exposure of the spindle to room temperature prior to electrofusion; the latter suggestion is surprising given that lowering the temperature leads to rapid spindle disassembly ([Pickering et al., 1990](#)), which apparently was the intention of the investigators. Yet, convincing proof that any of these approaches are safe for use in nuclear transfer in the human seems to be lacking. Using Sendai virus for spindle fusion, [Kang et al. \(2016\)](#) found 2 of 6 blastocysts to be euploid – an outcome rather similar to the results of [Zhang et al. \(2017\)](#). Thus the question of the best methodology for fusion of nuclei/spindles is still open to debate.

Mutation load and mutant mtDNA carryover

The authors did not test directly the enucleated oocytes of the patient for mutation load and only arrived at their conclusion that the mutation load was 100% through calculation. Given Kang and colleagues' report ([2016](#)) of a mutation load varying from 0.6% to 100% in tested cytoplasts from carrier patients, this is clearly a weakness of the experimental design by [Zhang et al. \(2017\)](#) and one that should be addressed in future attempts.

The blastocyst that was transferred to the patient showed a 5.7% mutation load. This is a higher carryover load than that reported by other groups, but it is unknown whether these differences could be partly due to differences in sensitivity and accuracy of the assays used in the different studies. [Tachibana et al. \(2009\)](#) reported nearly homoplasmic offspring following spindle transfer in the monkey, and [Kang et al. \(2016\)](#) obtained human embryos after spindle transfer with <1% mutant maternal mtDNA. However, the latter also report gradual loss of donor mtDNA and a full reversion to the maternal haplotype in embryonic stem cells derived from these embryos after multiple passages *in vitro*. Although 'reversion' is a significant concern, as [Zhang et al. \(2017\)](#) discuss, it is not known whether the

mechanisms underlying reversion in embryonic stem cells *in vitro* are also operative *in vivo* in humans. Thus long-term follow up of children resulting from MRT is crucial.

Outlook for the child

According to the authors, the child, who was 7 months old at the time of reporting, is healthy. The mutation load varied in the newborn baby's tissues, the lowest level of 2.36% having been found in the urine precipitate and the highest level of 9.23% in the circumcised foreskin. The level of heteroplasmy is a key issue in MRT since high levels can lead to manifestation of disease. The question of whether mutation load increases with increasing age is one that cannot be answered definitively at this point. Only follow up of this child and other children to be born from MRT will provide that answer. A survey-based follow up of 17 children, ranging in age from 13 to 18 years, born following cytoplasmic transplantation in the 1990s was recently published [Chen et al., 2016] and included two children in whom testing after birth had shown presence of both normal donor and maternal mitochondria [Barritt et al., 2001]. These children reportedly remain healthy [Chen et al., 2016] but it should be noted that repeated testing of the proportions of donor and maternal mitochondria in the young adults was not performed, consistent with the wishes of the families. Indeed, in the case reported here also, the family has requested that no further testing for mutant mtDNA load be undertaken, unless there is a clinical benefit.

Cross-border MRT?

In the UK, the Human Fertilisation and Embryology (HFE) Act of 1990 was amended in 2008 to allow for regulations to be passed that would permit techniques that prevent the transmission of serious mitochondrial disease due to deleterious mutations in mtDNA. In 2011, the UK Government asked the HFEA to examine the safety and efficacy of these techniques and in response the HFEA established a scientific panel, with broad-ranging scientific and clinical expertise, to examine the evidence [HFEA, 2011]. In addition, the HFEA was asked in January 2012 to carry out a public dialogue on the social and ethical impact of making these techniques available to patients [HFEA, 2012]. Two further panel reviews were carried out in 2013 and 2014 [HFEA, 2013, 2014]. In February 2015, following extensive debate, the UK House of Commons approved the enabling regulations, which came into force on 29 October 2015. The HFEA published a fourth panel report in November 2016 [HFEA, 2016a, HFEA, 2016b] recommending careful application of the technology to select patients. This recommendation was adopted by the HFEA on 15 December 2016, thereby enabling licensed fertility clinics in the UK to apply to the HFEA for a license to perform mitochondrial donation treatment. In addition, the Nuffield Council on Bioethics published a comprehensive report on the ethics of mitochondrial donation [Nuffield, 2012]. Thus, the UK has engaged in a deliberative and democratic – if perhaps unduly protracted – process, having taken 8 years to arrive at its current position.

In the USA, the picture is much less clear. In July 2001, prompted by the cytoplasmic transfer studies that took place in the 1990s [Cohen et al., 1997, 1998], the Center for Biologics Evaluation and Research (CBER) of the US Food and Drug Administration (FDA) exercised its

jurisdiction over 'cell and gene therapy products' and informed ART clinics of a requirement for an Investigational New Drug (IND) application for 'therapy involving the transfer of genetic material by means other than the union of gamete nuclei' [Johnson, 2016]. More recently, in a move toward approving MRT, and following an advisory committee's recommendations in 2014 [FDA, 2014], the FDA commissioned a report by the Institute of Medicine (IOM), the conclusions of which were positive toward MRT technologies and their purpose [Cohen and Adashi, 2016]. Most unfortunately, in December 2015, before publication of the IOM report, a federal statute, added to an appropriations bill, essentially prohibited the FDA from considering such applications, bringing progress in this area to a halt [Cohen and Adashi, 2016].

In the case described here, the MRT embryos were generated in New York through privately funded research, but the therapeutic part of the procedure – that is, transfer of the embryo to the patient – was done at an affiliated clinic in Mexico, thereby technically circumventing the federal statute. In response to questions raised by referees regarding the legal framework for their work, Zhang et al. [2017] shared with RBMO editors a pre-IND review request to the FDA, in which they described their past work and desire to continue to offer MRT to selected patients in the USA. However, bound by the December 2015 statute, the FDA apparently declined the investigators' request to meet or consider their application.

It is unfortunate that in this first attempt at MRT, investigators whose primary practice is in the USA found it necessary to resort to a clinic in a non-regulated jurisdiction. Such an action is open to criticism on many fronts, particularly the charge that it constitutes exploitation of vulnerable patients. Conversely, criticism of the use of the Mexican clinic lays one open to the charge of prejudice or ethical and cultural imperialism [Editorial, 2016], as it could rightly be argued that Mexico could have also passed laws against MRT had it wished to. Indeed, Palacios González [2016] has commented that the fact of this clinic having been used may favour the passage of very conservative legislation that is currently being considered by Mexico, but this outcome is not certain.

As outlined in a recent ASRM Ethics Committee opinion (ASRM, 2016), cross-border reproductive care (CBRC) is a growing reality in ART, motivated by a range of issues [Gürtin and Inhorn, 2011]. When the purpose of CBRC is to evade the law, some argue in favour of tolerance [Van Hoof and Pennings, 2011; Van Hoof et al., 2016]. Indeed, the European Court of Human Rights justified the use of CBRC within the EU as a way of circumventing national laws [European Court of Human Rights, 2010]. Thus, the criticism of Zhang et al. [2017] for proceeding in this way is at best muted.

Concluding remarks

The first successful case of MRT provides hope for the future of this technique and no one will take note of this success more than the patients who have been waiting in anticipation for the science to find itself solidly grounded and for the law to catch up with the science. The scientific community would do well in fulfilling its obligations to society in general, and to these patients in particular, by acknowledging the achievement of Zhang and colleagues while noting their work's shortcomings and providing constructive criticism for the benefit of future cases. The editors of RBMO hope that the publication of this paper, together with this accompanying editorial, marks a positive step toward that goal.

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