Towards an ethically acceptable proposal in the prevention of mitochondrial DNA-associated diseases

Lucía Gómez-Tatay, José Miguel Hernández-Andreu, Justo Aznar

Institute of Life Sciences, Catholic University of Valencia, Valencia, Spain

Corrispondenza: Justo Aznar, Institute of Life Sciences, Catholic University of Valencia, C/Guillem de Castro 94, 46003 Valencia, Spain, e-mail: justo.aznar@ucv.es

Ricevuto il 3 settembre 2015; Accettato il 18 dicembre 2015

RIASSUNTO

Le malattie dovute alle alterazioni del DNA mitocondriale (mtDNA) sono attualmente incurabili. C'è tuttavia un certo numero di metodi a vari livelli di sviluppo che potrebbe evitare la trasmissione madre-figlio di questi disturbi ereditari. Tra questi metodi ci sono due tecniche che sono attualmente attirando l'attenzione di ricercatori, decisori e pazienti: il trasferimento pronucleare (PNT) e il trasferimento del fuso materno (MST). Questi metodi comportano l'uso di mitocondri sani da un donatore. PNT ha luogo in uno zigote, mentre nell'MST, le cellule uovo sono manipolate. Questi metodi sono molto promettenti, in quanto sembrano essere efficaci e sicuri, e infatti, potranno presto essere utilizzati nella pratica clinica nel Regno Unito, dove sono già state elaborate alcune misure legislative. Tuttavia, queste tecniche, come sono concepite oggi, sollevano diverse questioni etiche. In questo articolo, proponiamo una possibile soluzione per superare questi problemi, dando alle famiglie colpite la possibilità di concepire bambini sani. Noi crediamo che, allo stadio attuale di sviluppo, ci sono due tecniche, la MST e il Trasferimento di Vescicola Germinale o l'Iniezione di Vescicola Germinale (GVT o GVI), che combinati con una tecnica di riproduzione, come il trasferimento nelle tube di Falloppio di gameti (GIFT) o il trasferimento della cellula uovo nella parte prossimale della tuba di Falloppio (LTOT), potrebbe essere ampiamente accettati. Al contrario, il PNT senza fornire alcun beneficio in termini di efficacia o sicurezza, comporta ulteriori gravi difficoltà da un punto di vista etico. A nostro parere, la ricerca e la legislazione devono seguire questa linea, dal momento che numerose questioni etiche verrebbero superate senza compromettere efficacia e sicurezza.

ABSTRACT

Diseases due to alterations in mitochondrial DNA (mtDNA) are currently incurable. However, there are a number of methods in various stages of development that could avoid mother-child transmission of these hereditary disorders. Among them are two techniques that are currently attracting the attention of researchers, policymakers and patients: pronuclear transfer (PNT) and maternal spindle transfer (MST). These methods involve use of healthy mitochondrial from a donor. PNT takes place in a zygote, whereas in MST egg are manipulated. These methods are highly promising, since they seem to be effective and safe, and in fact, they could soon be used in clinical practice in the UK, where legislative steps have already been taken. Nevertheless, these techniques, as they are conceived today, pose several ethical issues. In this paper, we propose a possible solution to overcome these issues, while giving affected families the possibility of conceiving healthy children. We believe that, at the current stage of development, there are two techniques, MST and Germinal Vesicle Transfer or Germinal Vesicle Injection (GVT or GVI), which combined with a reproduction technique such as gamete intra-fallopian transfer (GIFT) or low tubal oocyte transfer (LTOT), could be widely accepted. In contrast, the PNT without providing any benefit to these in terms of efficacy or safety, entails more serious ethical difficulties. In our opinion, research and legislation must be conducted in this line, since several ethical issues would be overcome without compromising effectivity and safety.

Parole chiave: malattia mitocondriale, trasferimento del fuso materno, trasferimento pronucleare, tecnologia di replicazione mitocondriale, procreazione assistita.

Keywords: mitochondrial disease, maternal spindle transfer, pronuclear transfer, mitochondrial replacement technology, assisted human reproduction.

1. Introduction

On 3rd of February of 2015, British Parliament voted in favor of mitochondrial transfer techniques, which can be used to prevent transmission of mitochondrial diseases from mother to child. While these techniques represent a major medical advance, they also raise objective ethical difficulties. In this paper, we analyze these issues.

Mutations in mitochondrial DNA (mtDNA) directly affect the function of the oxidative phosphorylation (OXPHOS) system, responsible for cellular energy production, giving rise to different clinical phenotypes [1]. Currently, there is evidence of nearly 600 different mutations of mtDNA [2; 3] associated with various syndromes, some of which can be very serious and even lead to death of the individual [4]. These diseases affect around 1 person in 5000, while 1 in 200 healthy people are carriers of a pathogenic mitochondrial mutation [5].

At present, there is no cure for these diseases, which are exclusively maternally inherited. Thus, medical research is moving toward developing techniques in order to prevent their transmission to offspring [6]. In recent years, two new techniques have been developed specifically for this purpose: maternal spindle transfer (MST) and pronuclear transfer (PNT). Both seem to have great potential for application, and could soon be legalized in England.

The aim of this paper is to review these techniques in order to make an ethical assessment of them. In addition, other techniques proposed in this field were considered to identify those options that did not raise ethical issues or, alternatively, propose another possibility that can be more widely accepted.

2. Mitochondrial donation techniques

MST and PNT are grouped under the term “mitochondrial donation”, as they involve the exchange of inherited mitochondria from the mother with others from a healthy donor. However, when making this exchange, there are some technical differences that will be very relevant to the ethical evaluation of these techniques.

2.1 Pronuclear transfer

This technique consists of performing *in vitro* fertilization (IVF) using eggs from the prospective mother, whose mitochondria contain mutated mtDNA, and sperm from the future father. Subsequently, after day one of embryo development, the pronuclei are removed, leaving most of the mutated mitochondria. These pronuclei are transferred into an enucleated zygote, formed by the union of a healthy donor egg and sperm from the future father or a donor. Pronuclei have to be transferred to an enucleated zygote, not an egg, because the state of development must be the same. The hybrid zygote then develops *in vitro* to a suitable state to be transferred to the womb [6].

In comparison to tampering with unfertilized oocytes, handling zygotes results in less physical damage caused by micromanipulation, which represents a technical advantage. However, it also raises serious ethical issues [7].

PNT was first successfully applied in mice in 1983 [8], and although some experiments were subsequently carried out on human zygotes, none of the pregnancies went to term [6]. The clinical application of PNT has been reported only once, in China. A triplet pregnancy was achieved, but did not go to term [9]. In 2005, a group of researchers from the University of Newcastle (UK) obtained a license from the Human Fertilisation and Embryology Authority (HFEA) [10] that allowed them to apply the technique in embryos with an abnormal number of pronuclei, which had been donated as they were unable to be used in fertility treatments. By applying this technique in human zygotes, the group managed to eliminate more than 98% of maternal mitochondria [11], which in principle would be sufficient to prevent clinical manifestation of the disease [12] and its transmission to subsequent generations [13]. However, the researchers suggested that further experiments were warranted in other situations: for example, using normally fertilized embryos or oocytes from women with a high level of mtDNA mutation [11].

2.2 Maternal spindle transfer

In metaphase II, the last phase of oocyte maturation prior to fertilization, the chromosomes are grouped on one side of the oocyte forming the so called spindle complex. MST involves extracting chromosomes at this stage from the maternal oocyte (whose mtDNA has a mutation) and then transferring them to a healthy oocyte donor, whose spindle complex has been removed. The hybrid egg is fertilized *in vitro* and then transferred to the uterus of the mother [6].

This technique has the ethical advantage that unfertilized oocytes are handled, so no embryos are created for the sole purpose of using them in treatment.

MST was performed in primates (*Macaca mulatta*) in 2009. The birth of four healthy monkeys was achieved and maternal mitochondria were not found (assay sensitivity 3%) [14]. These were the first animals born after an MST procedure. The technique was later tested on human oocytes [15]. In this case, although 52% of zygotes were abnormally fertilized, the rest were able to develop to blastocysts and to produce stem cells, similar to controls. Abnormal fertilization appears to be due to premature activation of oocytes prior to fertilization. The researchers suggested that performing manipulations in a medium without Ca^{2+} or supplemented with MG132 could be a solution to this problem. Further studies are therefore required for the clinical application of this technique.

3. Ethical aspects of mitochondrial donation

These techniques have a number of bioethical issues in common. First, a major increase in the number of oocyte donors would be necessary for research and clinical application. In this regard, regulations would have to be implemented in order to ensure the welfare of the donor, through appropriate recruitment and support, and by limiting the number of donations per donor to avoid the negative effects of repeated ovarian hyperstimulation [6]. However, the most relevant ethical issues are related to the fact that these techniques involve genetic modification of the germline, and that children born after their application would have a genetic link with three people: their parents and the donor.

3.1 Genetic modification of the germline

Genetic modification of the germline is the introduction of foreign DNA in the gametes or in the early embryo, which will be transmitted to the children and future generations. Somatic gene therapy is generally accepted, since it does not alter the overall nature of the genome and is not transmissible to offspring; it is thus considered comparable to surgery [16]. Germline gene therapy, however, is more controversial. In this case, the risks of genetic modification are difficult to predict, which is compounded by the fact that any alterations will be transmitted to offspring. Another drawback is the inability of people born after the application of these techniques to give their informed consent [6]. Furthermore, genetic manipulation of the germline may be used for eugenic purposes.

The fact that PNT and MST are forms of germline genetic modification has been used as an argument against the research and application of these techniques [6]. However, there is no general consensus among researchers about including these techniques in the field of germline gene therapy, since it does not act on the nucleus [6]. Certainly, it is important to consider the difference between nuclear DNA (nDNA) and mtDNA. It seems that mtDNA is only involved in the production of cellular energy and has no influence on the phenotype. Thus, its modification would not raise the same ethical concerns as modification of nDNA, which could alter essential characteristics and therefore the identity and personality of the individual [17]. Techniques based on germline modification to prevent transmission of mitochondrial diseases keep the nucleus intact. It is the mtDNA from a donor that will appear in the individual and in subsequent generations. Therefore, ethical restrictions on modification of nDNA do not apply. Nevertheless, some researchers who oppose these techniques argue that their acceptance could also lead to the approval of germline gene therapy in other cases, with unpredictable consequences [6]. However, the obvious difference between mtDNA and nDNA suggests that legalization of these techniques does not necessarily have to lead to the approval of germline nDNA modification [6].

Be that as it may, there is an objection to the establishment of a strict dichotomy between nDNA and mtDNA, as their interactions are still poorly understood. Thus, modifying mtDNA may influence the expression of nDNA [17]. In fact, one study suggests the involvement of mtDNA in cognitive functioning in mice [18], and an association between variation in mtDNA and susceptibility to alcoholism has also been found [19]. Therefore, ethical neutrality of mtDNA modification cannot be stated conclusively [17]. Studies must be conducted on the interactions between mtDNA and nDNA in order to determine whether mtDNA has some influence on our identity and personality and, if so, to what extent.

3.2 Recipient-donor relationship

Experiences of donors and recipients regarding the relationships established after donation, or the desire to contact the other party, vary depending on the nature of the donation [20].

Since mitochondria have their own DNA, their donation has different implications than the donation of organs or tissues, as conceived children have a genetic link with three people: their parents and the donor. There are few cases of children who have been born after mitochondrial donation treatment (i.e. cytoplasmic transfer, a different technique to PNT and MST which has been greatly discredited in the scientific community due to proven safety and effectiveness issues), and there is no evidence that these people have tried to establish any relationship with their donors and vice versa [6]. However, given the small number of cases, these data do not have great relevance. Thus, the implications for the child of being genetically related with three people remains a matter of speculation.

Nevertheless, given the low proportion of DNA provided by mitochondria (0.1%) [17], it does not seem reasonable to consider the donor as a third parent (or a second mother). It is undeniable that the same level of causality of parents themselves cannot be recognized in the donor, nor is it logical to define the donor as a motherless-mother. Therefore, this does not seem to be an appropriate term to refer to the donor. Furthermore, calling the donor "mother" could be harmful for the child, since it could affect the development of personal identity and perception of unity between their parents.

As regards the child's interest in contacting the donor or vice versa, this is something that could happen, as in other cases of donation of organs, tissues or gametes. Given that mitochondrial transfer techniques have been approved, legislation must consider this possibility, so that questions of confidentiality and possible contact with the donor are regulated in advance.

3.3 Ethical assessment of PNT and MST

Although these techniques share the ethical problems explained above, there are important differences between them that will be of great relevance to their ethical evaluation.

PNT poses serious moral impediments to its application, principally that it requires the destruction of one embryo for each embryo produced, since pronuclei have to be transferred to a "container" in the same state of development, i.e. an enucleated zygote. Moreover, its realization requires the production of embryos *in vitro*, since mitochondrial transplant takes place after fertilization, which requires access to the embryo.

MST does not raise these ethical problems, which are insurmountable for many. In MST, chromosomal transfer occurs prior to fertilization, so that oocytes are manipulated instead of zygotes. Moreover, unlike PNT, it can be considered independent of a subsequent IVF-embryo transfer (IVF-ET) procedure. Although in practice IVF takes place after ooplasm transfer, it does not have to be this way. As will be discussed later, there are other possibilities that allow fertilization to take place inside the mother's body. This is an important advantage for those who accept only those assisted reproductive techniques that merely assist and do not replace the procreative dimension of sexual intercourse.

4. Another possibility: Germinal Vesicle Transfer (GVT)

Immature oocytes, arrested in prophase I, are diploid (2n) and have the chromosomes within an intact nuclear membrane called the germinal vesicle. This technique involves removal of this vesicle from a patient oocyte and its transfer to an enucleated donor oocyte [21]. The resulting egg must be matured *in vitro*, for which there are currently no effective methods, thus greatly limiting the applicability of this technique [22]. Another drawback of this method is that, together with the germinal vesicle, a small amount of patient ooplasm containing mutated mitochondria is transferred to the donor oocyte, so that even after its application, the heteroplasmy clinical threshold could be exceeded.

However, a group of researchers has recently proposed a method called germinal vesicle injection (GVI) to prevent this transfer of ooplasm to achieve homoplasmy in the hybrid oocyte. It involves changing the traditional cell fusion method (electrofusion or inactivated Sendai virus (HVJ)) to a piezo-driven system which uses piezo pulses to inject the germinal vesicle into the oocyte [unpublished data, but presented at The 7th Conference of Asian Society for Mitochondrial Research and Medicine] [21].

This technique also involves germline manipulation. It now appears that MST or PNT could achieve a safe degree of heteroplasmy, i.e. less than the minimum clinical threshold, which cannot be said of GVT. However, a GVI variant could overcome this drawback. Nevertheless, we must also consider that in the present state of development of oocyte maturation techniques, the effectiveness of this method is limited, so that it is a less desirable option in terms of feasibility. Thus, GVT and GVI cannot currently compete against MST or PNT. Nevertheless, if technical difficulties were overcome, it could be a method to consider, as it is ethically equivalent to MST.

5. Alternative obstetric techniques

As explained above, both MST and GVT can be considered independently of the IVF procedure, which extends their possible range of acceptance to those sectors of society for whom IVF is not a morally acceptable option. Indeed, there are other techniques in the field of obstetrics that could be applied after MST, GVT or GVI treatment: gamete intra-fallopian transfer (GIFT) and low tubal oocyte transfer (LTOT).

5.1 Gamete intra-fallopian transfer

An alternative to IVF-ET is GIFT [23], a technique in which male and female gametes are transferred simultaneously, but separated by an air bubble, to the mother's body, where fertilization will occur. It is therefore an intracorporeal assisted reproduction technique. The ethical advantage of these techniques is that technical manipulation is prior to the formation of the zygote, as it is done on gametes.

However, for GIFT to be accepted by those who defend the unity between the procreative and the unitive moment, the latest has to occur, and the male gametes should be obtained immediately after completion of the conjugal act, by semen collection through vaginal washing. It is also necessary to make sure that the procreative moment occurs in the body of the mother and not during application of the technique [23].

The results of GIFT performed via vaginal transuterine transfer look promising, although available data diverge too much to talk about specific numbers. Compared to IVF, there are no significant differences regarding efficacy. While IVF-ET has a greater range of application to achieve pregnancy in infertile couples, this has no relevance in the field that concerns us, since women with mitochondrial disease would not, in principle, have any infertility problems.

5.2 Low tubal oocyte transfer

Another technique that could be applied in the context of preventing the transmission of disorders associated with mtDNA, and which has no ethical difficulties, is LTOT [23]. In this technique only eggs are transferred to the fallopian tube or uterus, via abdominal or transcervical transfer respectively, and then normal sexual intercourse takes place. However, the success rate is low (around 15%) and the literature on this technique is very scant, with only one document (dated 1980) found on a literature search conducted in Pubmed [24]. However, the effectiveness could probably be increased if resources were invested in the development of this technique. Furthermore, in this case of application, neither women nor their partners would have fertility problems, so that it is likely that it would be more effective.

6. Conclusions

Given the current inability to cure the disorders associated with mtDNA once they are present in the individual, preventing their transmission is presented as a promising possibility for families affected by these diseases, since affected women could conceive healthy children who shared their nuclear DNA. However, available techniques raise serious ethical concerns, as they involve a form of genetic modification of the germline, require the manipulation and destruction of many embryos and dissociate procreation from the sexual act. An alternative for those who perceive these ethical issues as insurmountable is the reintroduction of a manipulated egg, containing donated mitochondria, in the body of the mother, so that fertilization takes place thereafter naturally, and no embryos are destroyed.

MST, GVT and GVI are three possibilities that could be widely accepted, so that mitochondrial donation is prior to the formation of the zygote. However, for their clinical application to also be widely accepted, the subsequent IVF should be replaced by an assisted reproduction technique that preserves the life of the human embryo, as well as the connection between sexuality and procreation. Assisted reproduction techniques proposed for this aim are GIFT and LTOT, which allow the reintroduction of a manipulated egg in the mother's body.

Thus, we believe that application of MST, GVT and GVI should be legislatively promoted, since they may be conducted in an ethically acceptable manner and are a real possibility for preventing the transmission of mtDNA-associated diseases, although GVT and GVI techniques must be improved. We also believe that research efforts should not be divided between MST and PNT, since the latter, besides not presenting any advantage in terms of safety and efficacy, presents more ethical difficulties, insurmountable for many, that must be taken into account in the context of a pluralistic society.

References

[1] Chinnery PF, Hudson G. Mitochondrial genetics. British medical bulletin 2013; 106: 135-159.

[2] MITOMAP. A human mitochondrial genome database. MITOMAP: Reported Mitochondrial DNA Base Substitution Diseases: rRNA/tRNA mutations (retrieved on 29.5.2014, at: http://www.mitomap.org/bin/view.pl/MITOMAP/MutationsRNA).

[3] MITOMAP. A human mitochondrial genome database. MITOMAP: Reported Mitochondrial DNA Base Substitution Diseases: Coding and Control Region Point Mutations (retrieved on 29.5.2014, at: http://www.mitomap.org/bin/view.pl/MITOMAP/MutationsCodingControl).

[4] Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. Nature Reviews Genetics 2012; 13 (12): 878-890.

[5] Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF. Pathogenic mitochondrial DNA mutations are common in the general population. American journal of human genetics 2008; 83 (2): 254-260.

[6] Nuffield Council on Bioethics. Novel techniques for the prevention of mitochondrial DNA disorders: an ethical review. London; 2012.

[7] Klitzman R. The use of eggs and embryos in stem cell research. Seminars in reproductive medicine 2010; 28 (4): 336-44.

[8] McGrath J, Solter D. Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. Science 1983; 220 (4603): 1300-1302.

[9] Zhang J, Zhuang G, Zeng Y, Acosta C, Shu Y, Grifo J. Pregnancy derived from human nuclear transfer. Fertility and Sterility 2003; 80 (Suppl 3): 56.

[10] Human Fertilisation and Embryology Authority. HFEA grants licence to Newcastle Centre at LIFE for Mitochondrial Research. 2005 Sept 8 (retrieved on 12.3.2014, at: http://www.hfea.gov.uk/671.html).

[11] Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, Murdoch AP, Chinnery PF, Taylor RW, Lightowlers RN, Herbert M, Turnbull DM. Pronuclear transfer in human embryos to prevent transmission of mito-

chondrial DNA disease. Nature 2010; 465 (7294): 82-85.

[12] Hellebrekers DM, Wolfe R, Hendrickx AT, de Coo IF, de Die CE, Geraedts JP, Chinnery PF, Smeets HJ. PGD and heteroplasmic mitochondrial DNA point mutations: a systematic review estimating the chance of healthy offspring. Human reproduction update 2012; 18 (4): 341-349.

[13] Samuels DC, Wonnapinij P, Chinnery PF. Preventing the transmission of pathogenic mitochondrial DNA mutations: can we achieve long-term benefits from germ-line gene transfer? Human Reproduction: Oxford Journals 2013; 28 (3): 554-559.

[14] Tachibana M, Sparman M, Sritanaudomchai H, Ma H, Clepper L, Woodward J, Li Y, Ramsey C, Kolotushkina O, Mitalipov S. Mitochondrial gene replacement in primate offspring and embryonic stem cells. Nature 2009; 461 (7262): 367-372.

[15] Tachibana M, Amato P, Sparman M, Woodward J, Sanchis DM, Ma H, Gutierrez NM, Tippner-Hedges R, Kang E, Lee HS, Ramsey C, Masterson K, Battaglia D, Lee D, Wu D, Jensen J, Patton P, Gokhale S, Stouffer R, Mitalipov S. Towards germline gene therapy of inherited mitochondrial diseases. Nature 2013; 493 (7434): 627-631.

[16] Miralles, A Aparisi. El Proyecto Genoma y la ingeniería genética desde la perspectiva de los derechos humanos. Universidad de Navarra. Centro de Documentación de Bioética. 1999 (retrieved on 25.2.2014, at: https://www.unav.es/cdb/uncib2a.html).

[17] Bredenoord AL, Pennings G, de Wert G. Ooplasmic and nuclear transfer to prevent mitochondrial DNA disorders: conceptual and normative issues. Human Reproduction Update 2008; 14 (6): 669-678.

[18] Roubertoux PL, Sluyter F, Carlier M, Marcet B, Maarouf-Veray F, Chérif C, Marican C, Arrechi P, Godin F, Jamon M, Verrier B, Cohen-Salmon C. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. Nature genetics 2003; 35 (1): 65-69.

[19] Lease LR, Winnier DA, Williams JT, Dyer TD, Almasy L, Mahaney MC. Mitochondrial genetic effects on latent class variables associated with susceptibility to alcoholism. BMC Genet 2005; 6 (Suppl 1): S158.

[20] Nuffield Council on Bioethics. Human bodies: donation for medicine and research. 2011 Oct. (retrieved on 21.3.2016, at: http://nuffieldbioethics.org/wp-content/uploads/2014/07/Donation_full_report.pdf).

[21] Yabuuchi A, Beyhan Z, Kagawa N, Mori C, Ezoe K, Kato K, Aono F, Takehara Y, Kato O. Prevention of mitochondrial disease inheritance by assisted reproductive technologies: Prospects and challenges. Biochim Biophys Acta 2012; 1820 (5): 637-642.

[22] Bredenoord AL, Dondorp W, Pennings G, De Wert G. Nuclear transfer to prevent mitochondrial DNA disorders: revisiting the debate on reproductive cloning. Reproductive biomedicine online 2011; 22 (2): 200-207.

[23] Sgreccia, E. Personalist Bioethics. Foundations and applications. Philadelphia: The National Catholic Bioethics Center; 2012: 491-493.

[24] Kreitmann O, Hodgen GD. Low tubal ovum transfer; an alternative to in vitro fertilization. Fertil Steril 1980; 34 (4): 375-378.