Human Reproduction Update, Vol.14, No.6 pp. 669–678, 2008 doi:10.1093/humupd/dmn035
Advance Access publication September 4, 2008

Ooplasmic and nuclear transfer to prevent mitochondrial DNA disorders: conceptual and normative issues

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BACKGROUND: Mitochondrial DNA (mtDNA) disorders are an important cause of human diseases. In view of the limitations of prenatal diagnosis and preimplantation genetic diagnoses, alternatives such as ooplasmic transfer (OT) and nuclear transfer (NT) have been proposed to prevent the transmission of mtDNA mutations. Both OT and NT are radical in the sense that they do not entail genetic selection, but genetic intervention to correct the genetic cause of the disease. METHODS: After interviews with experts in the field, the relevant literature was searched and analyzed. Bioethical issues were divided into conceptual and normative points. RESULTS: OT is the transfer of normal mitochondria to a carrier's oocyte containing mutant mtDNA. In case of NT, a donated oocyte is enucleated and replaced with the nuclear DNA from a woman carrying a mtDNA mutation. NT can be performed both before and after *in vitro* fertilization, respectively, with the nucleus of an unfertilized oocyte, with the pronuclei of the zygote, or with the nucleus of a blastomere of an embryo. Conceptual questions regard whether these techniques amount to germ-line modification and human cloning. Normative questions concern, among others, the significance of intervening in the mtDNA, the implications of having 'three genetic parents', the ethics of oocyte donation and the health and safety risks for children conceived as a result of one of these techniques. CONCLUSIONS: Further interdisciplinary debate and research is needed to determine whether a clinical application of OT and NT can be morally justified, and if so, under which conditions.

Keywords: ethics; genetic disorders; mitochondria; nuclear transfer

Introduction

The Human Fertilization and Embryology Act (1990), one of the world's first laws enacted to regulate developments in assisted reproductive technology and embryo research, is currently under review. In December 2007, the UK House of Lords discussed a special amendment of the law, containing a proposal to permit pronuclear transfer to prevent mitochondrial DNA (mtDNA) disorders. The heated political debate focused on the acceptability of germ-line modification, the fear of reproductive cloning and the significance of the mtDNA. Earlier, in 2005, the Human Fertilization and Embryology Authority (HFEA) granted a license to determine the feasibility of pronuclear transfer (Brown *et al*., 2006). This license also evoked public and political attention (e.g. Randerson, 2004). Pronuclear transfer is not the only strategy that has been proposed as a possible preventive option for women at risk of transmitting a mtDNA mutation to their offspring.

Mitochondrial disorders can both arise from nuclear gene mutations and from defects in the mtDNA. MtDNA mutations are an important cause of human diseases, although the precise prevalence is difficult to estimate (Taylor and Turnbull, 2005). Mitochondrial disorders usually are severe disorders, involving defects in energy production. Although mtDNA disease may present 'with any symptom in any organ at any age' (Munnich *et al*., 1996; Haas *et al*., 2007), often the most energy demanding tissues such as the central nervous system, heart and skeletal muscles, liver and kidneys are affected. Because there is no curative treatment (Taylor and Turnbull, 2005; Chinnery *et al*., 2006), the prevention of the transmission of mtDNA disorders is considered to be of key importance. Although prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) are morally acceptable in specific circumstances, the application of both techniques for mtDNA disorders raises complex ethical questions (Bredenoord *et al*., 2008a,b). In view of the limitations of PND and PGD, the scientific community is searching for 'radical' alternatives, including ooplasmic transfer (OT) and (pro)nuclear transfer (NT). These approaches are radical in the sense that they do not entail genetic selection, but genetic intervention (or modification) to correct the genetic cause of the disease. These techniques aim to avoid mitochondrial disorders by intervening at the very beginning.

As preclinical experiments are currently performed and the clinical application of pronuclear transfer may be seriously

considered in the near future, this paper proactively identifies the ethical issues involved. After interviews with experts in the field, we searched and analyzed the relevant literature (using a so-called snowball method). Bioethical issues were divided in conceptual and normative aspects of OT and NT. Our aim is to clarify these complex conceptual and normative aspects and to scrutinize both the ethical questions surrounding the development of those techniques in the laboratory as well as their possible clinical application. The main part of this paper will focus on NT (instead of OT) for three reasons: first, NT seems more promising, secondly, recently doubts have risen regarding the value of OT in patients with mtDNA disease (Brown *et al*., 2006) and finally, the conceptual and normative questions for both techniques largely overlap.

Ooplasmic transfer

In the literature, both the terms ooplasmic transfer and 'cytoplasmic' transfer are being used. In this paper, we use the more precise term ooplasmic transfer. This is the transfer of donor ooplasm with normal mitochondria to an oocyte containing mutant mtDNA. It has been introduced as an assisted reproductive technique for women who experienced repeated embryonic development failure. As defective ooplasm may cause infertility, normal mitochondria from a donor oocyte are transferred into the patient's oocytes (Cohen *et al*., 1997, 1998; Huang *et al*., 1999; Lanzendorf *et al*., 1999; Barritt *et al*., 2001a,b; Krey *et al*., 2001; De Wert, 2003; Jacobs *et al*., 2006). This may provide the mitochondrial 'boost' necessary for embryonic development to prevent IVF failures (Van Blerkom *et al*., 1998). OT would also dilute or reduce the effect of mtDNA defects (Kagawa and Hayashi, 1997). However, the suitability and feasibility of OT for preventing mtDNA disease has been doubted for two reasons. First, in the initial applications in women experiencing repeated embryonic development failure, a relatively high number of chromosomal abnormalities and birth defects after OT have been reported (Jacobs *et al*., 2006; Brown *et al*., 2006). Secondly, in those applications only small amounts (10–15%) of donor ooplasm were transferred to the recipient's oocyte. To prevent the transmission of mtDNA disease, a larger amount (up to 50%) of donor ooplasm is needed. It is seriously questioned whether it is possible to introduce such an amount of ooplasm into the oocyte. The consequence is that the relative proportion of mutated to wild-type mtDNA is unlikely to change enough to prevent clinical disease (Thorburn and Dahl, 2001; Jacobs *et al*., 2006; Taylor and Turnbull, 2005; Brown *et al*., 2006; Fulka *et al*., 2007; Gardner *et al*., 2007).

Nuclear transfer

Although the technique was proposed earlier, it was only in 1999 that Roberts announced that 'a new human reproductive option now has the potential to eliminate the possibility of recurrence of mitochondrial disease in affected families' (Rubenstein *et al*., 1995; Roberts, 1999:265). In case of NT, a donated oocyte is enucleated and replaced with the nuclear DNA from a woman carrying a mtDNA mutation. As the woman's female relatives could be carriers as well, the donated oocyte would preferably be from the paternal side of the family or from an unrelated donor. The nuclear transplantation can be performed both before and after *in vitro* fertilization (IVF), respectively, with the nucleus of an unfertilized oocyte, with the pronuclei of the zygote (biopsied during the fertilization process), or with the nucleus of a blastomere of a fertilized oocyte, i.e. an embryo (De Wert, 2000; Brown *et al*., 2006). The current studies in the UK regard the feasibility of this second variant of NT, also called pronuclear transfer (Brown *et al*., 2006; Gardner *et al*., 2007).

Conceptual issues

Is OT and/or NT a type of germ-line modification?

A first conceptual issue concerns whether OT and/or NT should be classified as forms of germ-line modification. This question can be answered both affirmatively and negatively, depending on what is determined as the defining characteristic of germ-line modification. In general, a modification of the germ-line means that new genetic material is introduced into the gametes (or early embryo). This genetic modification is not only passed on to the child, but also to subsequent generations. In this sense, both OT and NT can be perceived as forms of germ-line modification. The mtDNA of the oocyte of the affected mother is supplemented with (OT) or exchanged for (NT) mtDNA of a donor oocyte and therefore irreversibly changed (Rubenstein *et al*., 1995; De Wert, 1999; Health Council of the Netherlands, 2001; Thorburn *et al*., 2001), although it can be questioned whether the small amount of mitochondria introduced in case of OT will pass to the next generation.

Initially, germ-line modification was synonymous with changes in the nuclear DNA. When the definition of germ-line modification is restricted to modification of the cell 'nucleus', then OT and NT would not amount to germ-line modification. After all, the cell nucleus remains intact. Both OT and NT to prevent mtDNA disease can be classified as a therapeutic germ-line intervention (or modification), whereby only the cell membrane is penetrated but the nuclear membrane is not (Wivel and Walters, 1993). They encompass a type of germ-line modification in which only the mtDNA is changed, which is extra-nuclear DNA.

Clearly, this conceptual issue is highly relevant for the normative analysis of OT and NT. After all, human germ-line modification is ethically much more controversial than somatic genetic modification. It is prohibited by international organs, such as the Council of Europe (Council of Europe, 1997 Art. 13: 'an intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants'). Some countries, though, legally make a distinction between germ-line modification of the nuclear DNA and germ-line modification of the mtDNA. The Dutch Embryo Act (2002), for example, leaves room for modifying the mtDNA, while modification of the nuclear DNA is prohibited. Also the HFE Act 2007 review proposes to prohibit modifications of both the nuclear and the mtDNA 'with an exception' for modifications of the mtDNA that are designed to prevent the transmission of serious mitochondrial disease.

The question is whether the strict dichotomy that is made between nuclear DNA and mtDNA is tenable. Much, after all, is unknown about nucleo-mitochondrial interaction (St John *et al*., 2004; Poulton *et al*., 2006; Bowles *et al*., 2007). Modifications

in the mtDNA may also influence nuclear gene expression. What does it mean to alter the match between nuclear and mitochondrial complements (Bonnicksen, 1998)? The ethical assessment of germ-line modification (in the context of mtDNA disorders) should therefore also reflect on the significance of the distinction between nuclear DNA and mtDNA.

Is nuclear transfer a type of human cloning?

The second conceptual issue, relevant for NT only, is whether it amounts to human cloning. This question also can be answered both affirmatively and negatively, depending on what is determined as the defining characteristic of cloning and on whether fertilized or unfertilized oocytes are used (De Wert, 2000; Jacobs *et al.*, 2006). Usually, cloning is defined as the production of a genetic copy, a genetically identical organism or individual ('genetic duplication'). Some may deny that NT is a form of cloning because this application is not aimed at the conception of genetically identical individuals but at the prevention of severe genetic disorders. But what is, or should be, the essential or defining characteristic of cloning? Is it the aim, the technique and/or the results?

As there is much confusion about the terms used in the debate on human cloning (President's Council on Bioethics, 2002), a further clarification of the concept is needed for the moral evaluation. Several distinctions and definitions have been proposed (De Wert, 2000). A first distinction is between embryo cloning (when the original cell is derived from an embryo) and adult cloning (when the original cell is derived from an adult). A second distinction is between reproductive cloning and non-reproductive cloning. Whereas reproductive cloning aims at the conception of a child, non-reproductive cloning aims primarily at obtaining stem cell lines for research. It is therefore also called 'therapeutic cloning'. This latter distinction has, however, been criticized from two different sides. A first criticism comes from those who object to the distinction between reproductive and non-reproductive cloning, because all cloning would be reproductive. After all, so they argue, all human cloning entails the production of a cloned human embryo. This group prefers to use instead the terminology 'cloning to produce children' and 'cloning for biomedical research' (President's Council on Bioethics, 2002). A second criticism comes from those who argue that cloning should exclusively be reserved for 'cloning to produce children'. Instead of cloning for research, they use 'somatic cell nuclear transfer' (SCNT) or 'nuclear transplantation'. This is mainly inspired by the wish to avoid the negative connotations that stick to the term cloning. This distinction, in turn, is criticized because it only describes the technique but fails to convey the nature of the deed itself (President's Council on Bioethics, 2002).

Clearly, no consensus exists regarding the adequate terminology. Taking into account the preceding discussion, we use in this paper the following classification of human cloning:

(i) Research cloning: all applications where cloning is used for research.
(ii) Reproductive cloning: all applications where cloning results in a new human being/individual.
    a) Reproductive embryo cloning: when the original cell(s) is derived from an embryo.
    b) Reproductive adult cloning: when the original cell(s) is derived from an adult human being.

Particularly 'reproductive' cloning is highly controversial and prohibited both by international organizations and by many national laws. The United Nations Declaration on Human Cloning (1998) calls upon member states to prohibit all forms of human cloning and the Council of Europe's Additional Protocol on the Prohibition of Cloning Human Beings forbids 'any intervention seeking to create a human being genetically identical to another human being, whether living or dead' (Council of Europe, 1998).

Does NT to prevent mtDNA disease amount to human cloning, and if so, to what category? It is important to have a look at the technique of NT. After all, the nuclear transplantation can be performed with

(i) the nucleus of an 'unfertilized' oocyte (NT type 1).
(ii) the pronuclei of a 'half fertilized' oocyte (the zygote; i.e. the sperm has penetrated the oocyte but no fusion or syngamy has yet occurred) (NT type 2).
(iii) the nucleus of a 'blastomere' of an embryo (NT type 3).

The first and second type, where either the nucleus of an unfertilized oocyte or the pronuclei of a zygote are transplanted, do not amount to human cloning, because no genetically identical twins are created. No duplication occurs. The second and third strategies differ only minimally in time. The period where the oocyte is 'partly fertilized', but the genetic material is not yet fused (the so-called presyngamy stage) constitutes only a thin, but significant line. The third type, where the nucleus of a blastomere is transplanted, does constitute human cloning, more specifically reproductive embryo cloning (De Wert, 2000). The fact that the new embryo has different mtDNA and thus is not entirely identical to the original embryo does not change this conclusion. After all, cloning is usually defined as sharing identical nuclear DNA (Cohen and Tomkin, 1994; Council of Europe, 1998; Health Council of the Netherlands, 2001). By the way, NT type 3 does not necessarily result in the birth of genetic identical individuals. This depends on the number of embryos transferred to a womb and subsequently on the number of children thus created (this will be discussed below).

The two conceptual questions regarding germ-line modification and cloning show that definitions and the choice of a specific type of technique raise different moral questions. Germ-line modification is at least as controversial as human cloning. Questions as whether modifications in the mtDNA should be the exception to the ban on germ-line modification (such as in Dutch law) or whether 'mitochondrial disease pose[s] a unique ethical argument for human cloning' (Roberts, 1999:265), demonstrate that the prevention of mtDNA disease by means of these technologies raises puzzling issues. We will now turn to the normative issues surrounding both OT and NT.

Normative issues

The moral value of embryos

Embryo research is required in the preclinical phase for the development of nearly every new reproductive technique. This is the

case for OT, and this also applies to the development of NT. The aim of the preclinical studies is to check the safety and feasibility of NT and to make a preclinical risk assessment (which is discussed below). The use of embryos for research is unacceptable for those ascribing high or absolute moral status to embryos. According to this position, all wastage of embryos is dismissed, the discarding of supernumerary embryos after IVF included. For those ascribing low or no independent moral status to embryos, embryo research is justified if 'for good reasons' and on the condition that the providers of the gametes gave their consent. In many experiments embryos left over after IVF can be used, but for the development of NT it may be necessary to create embryos especially for research as well. Creating embryos for research is rather controversial. It is also suggested that creating human-animal cybrids may provide the opportunity to learn more about the biology of mitochondria (St John and Lovell-Badge, 2007). Also this practice is controversial.

Although embryos are needed in the preclinical phase, a moral advantage of NT over other strategies, such as PGD and PND, could be that eventually no embryos or fetuses are lost—it may, in the end, be 'embryo-saving'. After all, the whole rationale behind PGD is embryo selection. The embryos not selected for transfer will be discarded. In case of PND, an adverse test result may lead to a termination of pregnancy. NT type 1 on the contrary only uses oocytes and NT type 2 only sperm and oocytes. NT type 3 would in theory only use one embryo (although in practice probably more embryos would be created, mainly because of the inefficiency of the technique). Whether it is acceptable to use embryos as a means to save embryos and fetuses in the long run depends, again, upon one's view of the status of the embryo.

In their experiments on NT type 2 (pronuclear transfer), the Newcastle researchers use abnormally fertilized human embryos (Gardner *et al*., 2007). More precisely, they use tripronuclear zygotes leftover after IVF. Tripronuclear embryos clearly are not viable. This makes (provided one ascribes low status to embryos) their use ethically less problematic: they would not have been eligible for transfer, and thus would be discarded anyway. Although the use of tripronuclear embryos may not be that problematic, using such embryos does lead to a puzzling conceptual issue: what exactly constitutes an embryo? The Dutch Embryo Act (2002) defines an embryo as a cell or cluster of cells with the potential to develop into a human being (art 1c). Clearly, according to Dutch law, non-viable embryos such as tripronuclear zygotes are not embryos. In theory, researchers thus can use them for whatever reason they like and without ethical review. This seems counterintuitive and a gap in the law (De Wert, 2001; Dondorp and De Wert, 2005; Olsthoorn-Heim *et al*., 2006). Likewise, when the embryo is defined as a fertilized oocyte, then the resulting 'organism' of all techniques in which other 'modes of production' are used (such as somatic cell nuclear transfer, i.e. cloning) would, strictly speaking, not be embryos either. That may lead to counterintuitive conclusions as well. To the extent that this organism would be capable of growing into a human being it would have the same claim to protection as do embryos resulting from fertilization (Dondorp and De Wert, 2007). The question, therefore, that urges on further reflection is: what makes something a human embryo (e.g. Devolder, 2006)?

Health and safety risks

The introduction of OT in the clinic has been a controversial process. In the initial applications of OT for women experiencing repeated embryonic development failure, a relatively high number of chromosomal abnormalities and birth defects has been reported (Jacobs *et al*., 2006; Brown *et al*., 2006), but it is unclear whether this is related to the technique of OT (Barritt *et al*., 2001b). Commentators criticized the premature introduction of OT in the clinic. They pointed at the absence of basic preclinical research and suitable experimental controls. They furthermore argued that, when applying 'pioneering' methods, the safety and efficacy should be evaluated in animal models first, followed by a public discussion and ethical review (e.g. De Wert, 1999; Parens and Juengst, 2001; Hawes *et al*., 2002). Subsequently, the Food and Drug Administration declared in 2001 that OT and related protocols are subject to formal review and approval (Zoon, 2001). It was emphasized that before widespread application is considered, conducting animal research and (pre)clinical trials is important to address the unanswered questions regarding both the efficacy and safety of the technique (Templeton, 2002; Brenner, 2004). It is, by the way, questioned whether obtaining such review and approval will be feasible: 'the controversial nature of such human gamete manipulation has resulted in a premature *de facto* ban on the clinical trials necessary to address these issues' (Malter, 2002:119).

Preclinical scientific research to assess the feasibility and safety of NT is currently being performed with (tripronuclear) embryos left over after IVF (see above). When NT proves to be successful and effective in preclinical feasibility studies, the step to the clinic may one day be considered. Some argue that huge problems need to be overcome before entering into the clinical phase may be considered (Poulton *et al*., 2006).

Regarding both OT and NT, two possible health risks are emphasized. First, it is unknown whether a mixture of mtDNA from two different origins is safe (Thorburn *et al*., 2001). In the case of NT, small amounts of affected mtDNA may come along with the nucleus or pronuclei, resulting in mtDNA heteroplasmy (Spikings *et al*., 2006; Bowles *et al*., 2008). Obviously, it is important to know the amount of mtDNA that may come along. If the amount of mutant mtDNA would exceed the threshold to disease expression, then NT may overreach its goal. In the case of OT, there is an inherent mixture of mtDNA. Evidence of mtDNA heteroplasmy was present in several children born after OT (Brenner *et al*., 2000; Barritt *et al*., 2001a,c). Although some bring to the fore that there is no reason to consider the minimal proportion of detected donor mitochondria observed in the offspring as harmful (Barritt *et al*., 2001a), the effects on children are unknown. Second, much is still unknown about epigenetic factors such as nucleo-mitochondrial interaction (St John *et al*., 2004; Poulton *et al*., 2006; Bowles *et al*., 2007). What will happen in the case of NT when the mtDNA is replaced? Some argue that scientific knowledge is insufficient to assess the risk/benefit ratio of NT (Szebik, 1999).

Although determining acceptable health risks to mother and future child is a tricky enterprise for all new reproductive technologies, a complicating factor is that NT generates irreversible changes. Whatever effect is produced, it will be passed on in the germ-line to successive generations. Sufficient knowledge about

the possible risks is therefore even more important. Article 5a of the United Nations Universal Declaration on the Human Genome and Human Rights (1997) states that 'research, treatment or diagnosis affecting an individual's genome shall be undertaken only after rigorous and prior assessment of the potential risks and benefits pertaining thereto'. But how to put this into practice? The introduction of IVF and ICSI showed that the safety of assisted reproductive technologies can only be proven after many years. How to adequately assess the potential risks and benefits in order to govern new reproductive technology? A principle often referred to is the Precautionary Principle. Although this principle comes in many versions, the strong version counsels that we should refrain from acting until safety is established through clear evidence: 'better safe than sorry' (Sunstein, 2002–2003) (or the Hippocratic prescription: *in dubio abstine*). Where some argue that taking precautions makes good science (e.g. Howard and Saunders, 1999), others argue that it stifles discovery or paralyzes scientific and technical progress (Harris and Holm, 1999; Sunstein, 2002–3). To govern the introduction of new reproductive technology one could also adhere to a 'proof first' approach, placing the burdens on the regulator to demonstrate a high risk of serious harm (Harris, 2007). Intermediate approaches may be conceivable as well.

Most if not all will agree that a decent minimum of safeguards for the health of the children is morally required—or perhaps even the first consideration (Peters, 2004; President's Council on Bioethics, 2004). Applying new technique without those safeguards resembles the premature introduction of new drugs without proper research (Pennings *et al.*, 2007b). The question of course is when 'enough' safeguards are made to justifiably introduce experimental reproductive and genetic technologies in general, and OT/NT in particular, in the clinic. Furthermore, a related question to be addressed is whether (and if so under what conditions) the introduction of a potentially risky technique is justified when safe alternatives, such as oocyte donation, are at hand. This requires a determination of the weight assigned to genetic parenthood (Peters, 2004; Graumann and Haker 1998).

Ethical aspects of reproductive embryo cloning in the context of mtDNA disease

We concluded above that only NT type 3, transplantation of the nucleus of a blastomere, amounts to reproductive embryo cloning. Using the pronuclei instead of the nucleus of a blastomere was crucial for the research license given to Newcastle University (to perform experiments with pronuclear transfer) (Human Fertilization and Embryology Authority [HFEA] summary decision RO153). After an initial rejection, the HFEA in second instance approved the Newcastle protocol as it concerns transfer of the 'pronuclei'. Cloning is thus avoided. As mentioned above, reproductive cloning is prohibited both by international organizations and many national laws. The current HFE Act (1990) and Reproductive Cloning Act (2001) for example prohibit reproductive cloning. 'The Appeal Committee accepted the view that the zygote at no stage contains a single nucleus. First it has two haploid pronuclei, then two diploid pronuclei, and then it enters mitosis' (HFEA summary decision RO153). Because the pronucleus is not the same as the nucleus, the HFE Act does not prohibit research involving pronuclei.

Data may also indicate that pronuclear transfer is more efficient than transfer in other stages (Taylor and Turnbull, 2005). Let us, however, do a thought experiment: what if the transfer of the nucleus of a blastomere, NT type 3, would be more efficient and safer than the transfer of the pronuclei? A relevant question then is whether the moral arguments to avoid reproductive cloning would outweigh technical efficiency and thus, perhaps, better results. Suppose the choice is made for NT type 3 and the nucleus of a blastomere is transferred. This may cause a dilemma. On the one hand, the creation of cloned children can be avoided by using only one blastomere of an embryo. The other side of the coin, however, is that the remainder of the embryo will be discarded. Therefore, one might opt to 'save' this embryo by using all its blastomeres. Perhaps up to four (or even more) clones may thus be created. One may have to choose between limiting the loss of embryos and avoiding the creation of genetically identical children (De Wert, 1999). What should be preferred: avoiding reproductive cloning, saving blastomeres or reproductive efficiency?

The principal question is whether it is categorically wrong to (reproductively) clone human beings. The moral debate on reproductive cloning is rather complex. Provided it would be safe, there may well be some moral reasons in favor of cloning. It may, for example, be used as an assisted reproductive technique in case of infertility, or (as in our discussion) to avoid genetic disease (Brock, 1998). Furthermore, some argue that the onus is on the opponents to produce arguments that reproductive cloning would be harmful (Dawkins, 1998). Others, on the other hand, dismiss reproductive cloning because it causes a 'yuk reaction' (Midgley, 2000), or because it would be unnatural. Other critics of reproductive cloning argue that it would violate a child's right to an open future, a concept coined by Feinberg (1980). As there will be a substantial time gap between the start of both lives, the clone has to live in the shadow of its 'original' (Holm, 1998; Buchanan *et al*., 2000). This argument, however, does not hold true for embryo cloning, at least insofar as twins thus conceived have a simultaneous start in life; this is not different from twins in the natural course of events (Bonnicksen, 1995; De Wert, 2000). Although they start their lives as genetically identical twins, they also begin their biography at the same time. They will thus be in ignorance of the future choices of the other (Buchanan *et al*., 2000). However, what if out of one single blastocyst different embryos would be made, cryopreserved, and be transferred sequentially? In that case, a clone would, again, have a (much) older twin. This may affect the sense of freedom of this child, or its right to an open future. It may have the feeling that it knows too much about itself, although this argument has been criticized for its genetic determinism (Brock, 1998; Buchanan *et al*., 2000; De Wert, 2000).

In any case, NT type 3 seems ethically more complex than the other variants of NT. Although the ethical debate has shifted primarily to research cloning (for obtaining stem cell lines), the context of mtDNA disease shows that the ethical debate on reproductive cloning remains important as well.

Germ-line modification: the moral implications of modifying the mtDNA

Another complex issue regards the debate on germ-line modification. Different types or arguments, both in favor and against,

have been put forward. Consequentialist objections, for example, emphasize the impossibility to foresee the consequences of germ-line modification (Health Council of the Netherlands, 2001; Salvi, 2001). We already touched upon this when discussing the health and safety risks. Deontological objections bring to the fore that children born as a result of the intervention will not have the possibility to give consent. Furthermore, the child will inherit a manipulated genome, which can be perceived as a violation of its genetic integrity. Article 24 of the United Nations Universal Declaration on the Human Genome and Human Rights (1997) mentions that germ-line intervention is a practice that could be contrary to human dignity. However, human dignity is a controversial concept (Salvi, 2001), not further defined in the Declaration. Nevertheless, it is usually defined as having intrinsic worth and deserving respect. At the liberal philosophical level, human dignity is used to indicate that persons should always be treated as ends in themselves and never merely as means. Bearing this in mind, if germ-line modification is used to diminish suffering, would human dignity be adversely affected? One could even argue that human dignity is promoted, as modification of the human germ-line is the only means of preventing/treating a certain condition. If the goal of the germ-line modification is clearly therapeutic, and safety can be guaranteed, we may have good reasons to assume that this future person would also have given consent. He/she will probably not regret that its genetic integrity has been violated.

The ethical debate on germ-line modification focused entirely on altering the nuclear DNA. Neither OT nor NT would involve modification of the nuclear DNA. What about the ethics of modifying the mtDNA?

The general assumption is that mtDNA does not really constitute our genetic make-up; it does not influence our phenotype, as it only governs cellular energy production. Modification of essential or defining characteristics is considered to be ethically more problematic, because it determines one's identity or personality. Modifying the nuclear DNA is therefore often regarded more problematic than modifying the mtDNA. This is for example shown by the exceptions made in the Dutch Embryo Law and the proposals in the HFE Act (2007) review. It is also shown by the approval of the research license to the researchers of Newcastle University, where the Appeal Committee 'accepted that mtDNA is not associated with identity or predetermined characteristics of the individual' (HFEA summary decision RO153).

Whereas there seems to be a strong consensus that the mtDNA does not influences our 'character', little is known about the exact role and function of the mtDNA (Thorburn *et al*., 2001). One study suggests mtDNA involvement in cognitive functioning in mice (Roubertoux *et al*., 2003). Another study detects evidence of associations between mtDNA variation and susceptibility to alcoholism (Lease *et al*., 2005). How much certainty and evidence is there that mtDNA does not influence characteristics and physical appearance? It might be premature to conclude that modifying mtDNA is 'ethically irrelevant/neutral'. Some commentators note that we cannot exclude that the mtDNA is able to influence our individuality, since the mtDNA determines a part of the function of the mitochondria, and these on its turn influence energy production of the (neural) cells (Szebik, 1999). Similarly, others note that the mtDNA is usually ignored in policy debates about genetic engineering, 'on the basis of the weak assumption that it does not have significant phenotypic effects. But mitochondria do govern cellular energy production, and we are learning more about the downstream and far-reaching effects of that function on human physiology and (through the brain) on human behavior' (Parens and Juengst, 2001:397). Although this seems improbable in the case of mtDNA, there is always the possibility that a gene product has unexpected effects.

Clearly, further discussion on the acceptability of germ-line modification in general and modification of the mtDNA in particular is necessary before considering to introduce OT and/or NT for mtDNA disease in clinical practice.

The moral implications of having three genetic parents

OT and NT result in an embryo that inherits the vast majority of its DNA from the intentional mother and father, plus a proportion of mtDNA from the oocyte donor. To put this into perspective: the donor would contribute 0.1% of her DNA (the 37 mitochondrial genes) while the intentional parents contribute the remaining 99.90% of their DNA (~24 000 nuclear genes). Strictly speaking, this may lead to the birth of a child with three genetic parents: the intentional parents delivering the nuclear DNA and the donor passing on the mtDNA (Health Council of the Netherlands, 2001). What are the moral implications of having three genetic parents?

The moral implications particularly relate to the interests of the child and the implications for the family. Some would consider NT to be unnatural or undermining the traditional family. This, however, are familiar objections to new reproductive technologies. In the early days of assisted reproductive technology, the impact of collaborative strategies (gamete donation, surrogacy) on the welfare of the child was already questioned. As Robertson (1994:13) puts it: 'Laboratory manipulation of embryos, the splitting of gestational and genetic parenthood, and prenatal screening risk producing children who are physically or psychologically injured by the techniques in question. Of special concern is the impact of children of several sets of genetic and social parents, some of whom the child will never know, which arise in the collaborative use of gamete donors and surrogates'. Collaborative reproduction is always more problematic than natural reproduction, as it introduces a third party into the usual situation of two-party parenthood and the 'traditional' genetic, gestational and social unity of reproduction might be separated.

As different collaborative strategies in the context of reproductive medicine are currently broadly accepted, the main concern regarding—and the novelty of—OT and NT is the mixing of mtDNA and the insertion of mtDNA of a third party into the cell. Some argue that this splitting of genetic motherhood should be considered the main ethical issue in the debate about OT and NT (Robertson, 1999). The psychological, legal and social consequences of having three genetic 'parents' are largely unknown. Moreover, it is debatable whether the oocyte donor, contributing her mtDNA, should be considered as a 'genetic parent' at all (Mertes and Pennings, 2008).

Ethical aspects of oocyte donation in the context of mtDNA disease

OT and NT require collaboration from oocyte donors in both the preclinical phase and in possible future clinical applications. Oocyte donation raises the same ethical questions as assisted conception in general or sperm donation in particular, such as the meaning of family values, sexuality, parenthood, gender relations

and commodification (e.g. Cohen, 1996). However, as different assisted reproductive technologies are currently broadly accepted, we focus on another point of attention: the welfare of the oocyte donor. The first issue concerns genetic parenthood. Women donating oocytes for reproductive purposes in the context of mtDNA disorders will pass on their mtDNA to a child. However, given that oocyte donors normally donate both nuclear DNA and mtDNA, donation of the mtDNA will probably be more easily accepted (Robertson, 1999) and psychologically less burdensome.

A second issue concerns the risks and burdens of oocyte retrieval. The donating woman has to undergo ovulation induction and oocyte retrieval, like in normal oocyte donation (except for cases where an IVF patient donates some of her oocytes). Relevant issues are how the health and safety risks involved can be weighed properly, how autonomous decision-making can be guaranteed and how exploitation of women can be avoided (De Wert, 1999; Mertes and Pennings, 2007; Pennings *et al*., 2007a). It is proposed to treat women who donate oocytes for research purposes like other healthy research subjects in clinical trials (Mertes and Pennings, 2007). This proposal may be extended to those who donate oocytes for preclinical studies in the context of NT. Perhaps this could be the proper model for oocyte donation in the clinical phase as well. This depends on how the contribution of mtDNA is morally evaluated.

A third point concerns the difficult access to and the scarcity of donoroocytes. One could argue that, since oocytes are scarce, they should be used for reproductive purposes and not for research. Others may reply that the appropriate person to determine the destiny of oocytes is the donor herself. After all, some women will have fewer problems with donating oocytes for research than with donating for reproduction since they will not be confronted with a genetically related child.

Regardless of the purpose for which the oocytes are donated, one should pay attention to minimization of risks, avoidance of undue influence and guarantee of informed consent (De Wert, 1999; Mertes and Pennings, 2007; Pennings *et al*., 2007b).

Nuclear transfer: down the slippery slope?

In discussions about (bio)medical technology, the 'slippery slope' or 'thin end of the wedge' is an often-heard argument. Introducing or accepting a technology or application A that in itself is not morally problematic, would be problematic if doing so makes it impossible (logically or empirically) to avoid the subsequent introduction or acceptance of another technology or Application B that is morally unacceptable (van der Burg, 1992; Burgess, 1993; McGleenan, 1995; Health Council of the Netherlands, 2001). In the logical version of the slippery slope, accepting A deprives one of valid arguments to reject B. The empirical version entails the prediction that accepting A will lead to a climate of acceptance of B as well. In both versions, the message is that anyone who regards B as undesirable, should reject A (Lamb, 1988; De Wert, 2005). In the case of NT, at least three slippery slopes can be discerned.

The slippery slope towards germ-line modification of the nuclear DNA

This slippery slope argument runs as follows:

(i) Once germ-line modification of the mtDNA is accepted, eventually modification of nuclear DNA will be accepted.
(ii) Subsequently, once germ-line modification is accepted for therapeutic uses, it will lead to the application for non-medical uses, i.e. enhancement (Health Council of the Netherlands, 2001).

Possible arguments in favor of those concerns are that once germ-line modification is successfully applied, it may lead to a climate wherein other modifications of the germ-line indeed may be investigated. Furthermore, the line between medical and non-medical uses is a troublesome one. On the other hand, arguments to refute this concern may be that no technique is currently capable of genetically altering characteristics of future human beings. Besides this, it is not the nuclear DNA that is altered during the procedure of NT, but the mtDNA. This may yield less ethical controversy than altering the nuclear DNA, which is, as we have discussed above, generally perceived as the locus of our characteristics and personality traits. Finally, this slippery slope argument assumes enhancement is wrong. Suppose NT indeed leads to enhancement, the question remains whether enhancement indeed is categorically wrong and therefore should be prohibited at all times, under all circumstances.

The slippery slope towards reproductive cloning

The second variant of the slippery slope argument concerns a gliding scale from NT to reproductive cloning.

(i) Earlier, three types of NT were discerned, using an unfertilized, a half-fertilized or a fertilized oocyte (i.e. a blastomere of an embryo). Although the first and second types do not implicate cloning, some critics may argue they nevertheless should be prohibited: once they are accepted, they may well lead to the third type of nuclear transfer, which clearly is a type a reproductive embryo cloning.
(ii) Subsequently, reproductive embryo cloning may eventually lead to reproductive adult cloning.

This slippery slope argument assumes that human cloning, more in specific reproductive embryo cloning, is to be condemned. Again, the fundamental ethical question to be addressed is whether it would always be, categorically, wrong to (reproductively) clone embryos. Even if that would indeed be the case, then it still does not automatically follow that accepting NT type 1 or 2 will inherently lead to NT type 3. NT type 3, on its turn, does not automatically have to lead to reproductive adult cloning.

The slippery slope towards misuse of NT

The third slippery slope regards the use of NT by other groups and with other aims than the prevention of mtDNA disorders. It may for example be used as an assisted reproductive technique for older, perimenopausal women seeking to have children (Parks, 1996; Eisenberg and Schenker, 1997; Barnett and McKie, 2004). Also in this slippery slope a debatable assumption is made, namely, that perimenopausal women should not reproduce in this way.

Social justice in health care

Another principle that needs to be scrutinized concerns the fair distribution of scarce resources. Justice and equal access to health care are among the most urgent issues in genetics and assisted

reproductive medicine (Pennings *et al*., 2008). An argument against NT could be that mitochondrial diseases are rare, and mitochondrial diseases caused by a mutation in the mtDNA even more. NT therefore has a limited applicability and should not be given priority. Moreover, the development of the technique is time-consuming and expensive. Hence, it can be argued that it is inefficient and unfair to spent scarce health care resources on this type of research, even more when alternatives such as adoption and oocyte donation are available. Furthermore, although NT might prove to be successful in preventing the transmission of mtDNA disease, the mother (and other patients) will not benefit herself, as no curative treatment is available (Rubenstein *et al*., 1995). Money may be better spent on developing treatment, to help those people already suffering from a mitochondrial disease.

On the other hand, although the prevalence of mtDNA disease is incomparable with, for example cardiac disease, recent data identify mtDNA disorders as an important cause of genetic diseases (Taylor and Turnbull, 2005). The fact that not many people are known with mtDNA disorders may be related to the fact that there is no single hot-spot among the mitochondrial disorders. Furthermore, many take the position that providing assisted reproductive and genetic technology to people at high risk of an affected child respects both the principle of justice and the principle of avoiding harm (Pennings *et al*., 2008). If we continue the analogy with cardiac disease, one might add that NT offers chances at the very beginning of life. Contrarily, many therapies and research concerning cardiac disease are centered at the end of life. The net gain of NT may be considerable. Moreover, the development and experiments with NT may yield interesting spin-off effects.

This difficult issue cannot be solved within the scope of this paper. Even if one argues that innovative techniques like NT should be developed, this still leaves the issue of reimbursement unsettled. The proper allocation of resources is actually a much wider moral and political issue that is and will be on top of the political agenda in the coming years.

Conclusion

In this paper, we identified the complex conceptual and normative issues that arise with regard to OT and NT to prevent mtDNA disorders. The first conceptual question we addressed is whether these techniques amount to germ-line modification. We concluded that both OT and NT encompass a type of germ-line modification in which only the mtDNA is changed. The consequences and the ethical significance of modifying the mtDNA need further scrutiny. The second conceptual question we addressed is whether NT amounts to human cloning. This depends on whether fertilized or unfertilized oocytes are used. Transferring the nucleus of a blastomere (NT type 3) indeed constitutes reproductive embryo cloning, making it ethically more complex than the other variants of NT. Normative issues concern, among others, the moral value of embryos, the health and safety risks for children conceived as a result of one of these techniques, the implications of having 'three genetic parents', the ethics of oocyte donation and the proper allocation of scarce resources. Further interdisciplinary debate and research should determine whether it is morally acceptable to introduce OT and NT in the clinic, and if so, under which conditions.

Funding

This work was supported by a grant from the European Union sixth Research Framework Programme (the MITOCIRCLE project contract no. 005260).

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Submitted on May 8, 2008; resubmitted on June 25, 2008; accepted on July 23, 2008