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ARTICLE

Nuclear transfer to prevent mitochondrial DNA disorders: revisiting the debate on reproductive cloning

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Abstract Preclinical experiments are currently performed to examine the feasibility of several types of nuclear transfer to prevent mitochondrial DNA (mtDNA) disorders. Whereas the two most promising types of nuclear transfer to prevent mtDNA disorders, spindle transfer and pronuclear transfer, do not amount to reproductive cloning, one theoretical variant, blastomere transfer does. This seems the most challenging both technically and ethically. It is prohibited by many jurisdictions and also the scientific community seems to avoid it. Nevertheless, this paper examines the moral acceptability of blastomere transfer as a method to prevent mtDNA disorders. The reason for doing so is that most objections against reproductive cloning refer to reproductive adult cloning, while blastomere transfer would amount to reproductive embryo cloning. After clarifying this conceptual difference, this paper examines whether the main non-safety objections brought forward against reproductive cloning also apply in the context of blastomere transfer. The conclusion is that if this variant were to become safe and effective, dismissing it because it would involve reproductive cloning is unjustified. Nevertheless, as it may lead to more complex ethical appraisals than the other variants, researchers should initially focus on the development of the other types of nuclear transfer to prevent mtDNA disorders.

KEYWORDS: ethics, mitochondrial DNA disorders, nuclear transfer, reproductive cloning

Introduction

Mitochondrial DNA (mtDNA) disorders are usually severe disorders, caused by defects in energy production. Patients show a wide variety of symptoms, but generally the most energy-demanding tissues, such as the central nervous system, heart and skeletal muscles, liver and kidney, are affected. As there is no curative treatment, helping carriers of mtDNA mutations to have healthy children has been a central focus of attention (Taylor and Turnbull, 2005). One reproductive option to prevent the transmission of a mtDNA mutation from mother to child that is currently in preclinical

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development is nuclear transfer (or 'mitochondrial gene replacement'). In case of nuclear transfer to prevent mtDNA disorders, the mtDNA (which is located outside the nucleus, in the cytoplasm) is changed or replaced (Bredenoord et al., 2008a). This should result in healthy offspring with the nuclear genes of the parents, but without the mtDNA mutation (Gardner et al., 2007). Nuclear transfer can in theory be applied at different stages: before, during or after fertilization (de Wert, 2000; Brown et al., 2006; Roberts, 1999).

Germinal vesicle transfer

This would involve transfer of the germinal vesicle, removed from a recipient woman's immature oocyte, into an enucleated donor oocyte. Subsequently, the reconstructed oocyte will be matured and fertilized *in vitro* using a spermatozoon from the partner. The resulting embryo is then transferred to the prospective mother's womb.

Germinal vesicle transfer has not been applied clinically and is not considered to be one of the most promising types of nuclear transfer, particularly because its efficacy is doubted due to the poor developmental competence of in-vitro matured oocytes (Fulka et al., 2005; Taylor and Turnbull, 2005; Brown et al., 2006).

Spindle transfer

This would involve transfer of the chromosome–spindle complex, removed from a recipient woman's oocyte when the nucleus is undergoing the second division of meiosis, into an enucleated donor oocyte (Brown et al., 2006). Subsequently, the reconstructed oocyte will be fertilized using a spermatozoon from the partner. The resulting embryo is then transferred to the prospective mother's womb. As mature oocytes do not have a nuclear membrane, there was earlier scepticism about the safety of transfer at this stage (Brown et al., 2006). Recent studies, though, are promising. Tachibana et al. (2009) showed that spindle transfer is technically feasible in non-human primates: they transferred the chromosome–spindle complex of a mature oocyte to an enucleated donor oocyte, resulting in three thus-far healthy macaque infants, with minimal levels of carry-over of nuclear donor mtDNA.

Pronuclear transfer

This would involve transfer at the zygote stage (Brown et al., 2006; Craven et al., 2010). An oocyte of the prospective mother is fertilized using a spermatozoon from the partner, as well as a donated oocyte of a healthy woman (the oocytes have to be at the same stage). When the oocytes are 'half fertilized', the two pronuclei (distinct structures that become apparent after fertilization) are taken out of the donated zygote. Subsequently, the pronuclei of the intentional parents (containing their nuclear DNA) are transferred to the enucleated donor zygote. The resulting embryo is then transferred to the prospective mother's womb.

Studies suggest the safety and efficacy of pronuclear transfer in preventing the transmission of mutated mtDNA in a mouse model (Jenuth et al., 1996; Meirelles and Smith, 1997, 1998; Sato et al., 2005). A technical advantage is that during this stage the chromosomes are packed into the pronuclei, which would make it easier to collect and transfer them (Taylor and Turnbull, 2005). On the other hand, mitochondria surrounding the pronuclei may increase the amount of pathogenic mtDNA transplanted from the donor into the recipient zygote. Recent preclinical studies have shown that pronuclear transfer is feasible in human oocytes, resulting in embryos with minimal levels of carry-over of nuclear donor mtDNA (far below the threshold of disease expression) (Craven et al., 2010). A clinical application of pronuclear transfer has been reported once, resulting in a triplet pregnancy but no life birth (Zhang et al., 2003).

Blastomere transfer

The nuclear DNA of a donated oocyte from a healthy woman is removed. An oocyte of the prospective mother is fertilized using a spermatozoon from the partner. A blastomere of the resulting embryo is then transferred to the enucleated donor oocyte. Subsequently, the resulting embryo is transferred to the prospective mother's womb (Roberts, 1999).

Blastomere transfer has not been applied clinically. Some expect the success rate of nuclear transfer using a blastomere of an embryo to be much lower than nuclear transfer at the other stages, particularly because animal studies showed evidence of high heteroplasmy levels: the co-existence of mutant and normal mtDNA in an affected individual (Steinborn et al., 1998, 2000; Hiendleder et al., 1999; Ferreira et al., 2007). In addition, the resulting embryo may have a poor developmental competence (Roberts, 1999; Spikings et al., 2006), although higher rates of development are observed with embryonic-cell compared with somatic-cell nuclear transfer (Mitalipov et al., 2002). These technical impediments make whole blastomere transfer currently less suitable and promising than spindle or pronuclear transfer.

Although remaining technical and ethical difficulties need further attention, both spindle transfer and pronuclear transfer are promising future reproductive options for carriers of mtDNA mutations (Poulton et al., 2010; Poulton and Bredenoord, 2010). In addition, both these variants of nuclear transfer would not amount to reproductive cloning. On the contrary, blastomere transfer could. This type seems both technically and ethically the most challenging variant of nuclear transfer to prevent mtDNA disorders. The scientific community seems to avoid it, perhaps also in response to the fact that many jurisdictions have prohibited this variant because it may involve reproductive cloning. For example, when the Human Fertilisation and Embryology Authority (HFEA) had to decide on the research licence for experiments on pronuclear transfer, it explicitly mentioned that transfer of a nucleus of a cell of an embryo is prohibited (which, depending on the definition, could be perceived as reproductive cloning; see below) (HFEA summary decision RO153).

Notwithstanding the poor technical performance of blastomere transfer to prevent mtDNA disorders and its avoidance by scientists, this paper discusses the moral acceptability of blastomere transfer as a method to prevent