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COMMENTARY

The biological basis for defining bi-parental or tri-parental origin of offspring from cytoplasmic and spindle transfer

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Abstract The bi-parental genetic state is not a given after assisted reproduction. This is based on a biological definition of parentage that concerns generational inheritance of genetic material. Often three or more individuals may participate in artificial reproduction. Only cytoplasmic and spindle transfer can result in the genetic tri-parental state. All other forms involving three or more assisting persons with no heritable genetic contribution must be considered differently. Can a cytoplasmic donor be a biological parent based on a potential contribution of mitochondrial DNA to the offspring? — only if the mitochondrial DNA sequence can be traced back to the donor, a phenomenon which may not be very common. When considering spindle transfer for avoiding transmission of mitochondrial disease, all offspring is likely to be tri-parental.

KEYWORDS: bi-parental, definition of parenthood, heteroplasmy, mitochondrial disease, mitochondrial DNA, tri-parental

In unassisted human reproduction, the bi-parental state is the genetic norm. Modern assisted treatments, however, have obscured this norm by mediating participation of three (or more) individuals in the act of reproduction. Oocyte and sperm donation and gestational surrogacy are common examples. Despite the involvement of three, four or possibly even five individuals in some scenarios, the children resulting from assisted reproduction treatment are still considered to be bi-parental. This is based on a biological definition of parentage that specifically concerns generational inheritance of genetic material. It is in this context that the term 'tri-parental' has been used to describe children born following cytoplasmic (or ooplasmic) transplantation. The bi-parental natural norm involves inheritance of both oocyte-derived (mitochondria) and sperm-derived (centrosome) organelles. Here we argue that the term tri-parental is unrefined, simplistic and biologically inaccurate. Biological parenthood has been previously defined in genetic, gestational and post-natal terms (Johnson, 1999). There are socioeconomic, cultural and legal dimensions to parenthood as well, adding even more layers of complexity to this already intricate concept.

The tri-parental condition in biological textbooks and bibliographies is expressly associated with a form of bacterial conjugation where a plasmid in a helper strain assists the transfer of a plasmid from a second strain into a third strain (Wise et al., 2006). Investigators studying tri-parental mating and sexual activity do not ordinarily distinguish mitochondrial DNA (mtDNA) as a separate genetic entity from nuclear DNA nor do they evaluate DNA sequences. Clearly, these established definitions are not relevant in the debate on tri-parenthood during and after assisted reproduction treatment. In humans, 'tri-parental' implies inheritance of genetic material from three different individuals or three biological parents. The question with respect to cytoplasmic donation is whether a cytoplasmic donor

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can be considered a biological parent based on a potential contribution of mtDNA to the offspring.

The mitochondrial genome, because of its short length and relative simplicity, was sequenced years before the nuclear genome (Anderson et al., 1981). This genome has fewer than 17,000 base pairs and encodes 37 genes, all of which are involved in oxidative phosphorylation or mitochondrial replication. The coding regions of mtDNA are similar in all individuals. Each cell contains multiple copies of mtDNA, each copy having potentially a slightly different sequence — so-called heteroplasmy. Some of these sequences are mutations that adversely affect function. Overall, mitochondrial function is dependent on the ratio of normal to mutated mtDNA per cell. Mutation rates increase with age in all individuals, but cellular function is only affected when the balance of normal and mutated DNA is tipped in favour of the latter. Even in preimplantation embryos, in which mitochondria are inherited solely from the mother, mtDNA mutations are common (Barritt et al., 1999, 2000; Yesodi et al., 2002). Familial mitochondrial disease becomes manifest when the balance is severely skewed towards inherited mutated mtDNA. Ratios may vary greatly even between individuals with similar disease, but a 50% ratio is usually regarded as indicating a pathological condition. Complicating matters are the temporal and tissue variations, which have been shown to occur in the human and the mouse (Aiken et al., 2008).

The coding regions of mtDNA cannot be distinguished by sequencing alone, unless the non-coding hypervariable region is included in the analysis. Differences between hypervariable sequences allowed Barritt and co-workers (2001) to distinguish between donor and maternal (recipient) mitochondria in embryos, fetuses and babies resulting from cytoplasmic transfer. This study showed that the genomic sequences of some of the mitochondria in the offspring were derived from the ooplasm donor. At the time of the last analysis, three of 13 tested fetal blood samples were positive for mtDNA from the ooplasm donor, and two of 13 tested babies showed mtDNA sequences derived from the ooplasm donor and the mother, a condition also referred to as heteroplasmy. It is unknown whether those donor sequences have survived in those children who tested positive as infants as they have grown older. The other babies were homoplasmic with only mtDNA in blood derived from the mother. Heterogeneity of type of mtDNA in different tissues, including blood, of heteroplasmic children can be expected to be similar to the ratio of mutated and wild type mtDNA in mitochondrial disease and so blood can be considered representative of other tissues (Barritt et al., 2001; Larsson et al., 1990). Thus, even if one were to consider mtDNA inheritance to be equivalent to nuclear DNA inheritance, the tri-parental state of the children in question is not a forgone conclusion.

There are, however, a number of biologically important questions that can be raised with respect to these findings: (i) If the two children who tested positive for cytoplasmic donor mtDNA are tri-parental, are the 11 children who tested negative bi-parental? (ii) Should the small conserved mtDNA sequence be included in the genomic identity of an individual? and (iii) Does a hypervariable area produce physiologically heritable changes in an individual or does it just pass on from generation to generation, thereby resembling many non-coding sequences of the nuclear genome?

In **Table 1**, we explore the various dimensions of parenthood by examining genetic and sociological contributions of each individual (person) involved in natural

Table 1 Examples of parenthood as a function of biological and sociological contributions of each individual involved in natural and assisted procreation.

Person	*Gamete (nuclear)*	*Mitochondrial donor*	*Assisting person*	*Biological genomic*	*Socioeconomic*	*Two-person assisted reproduction*	*Three-person assisted reproduction*	*Tri-parental*
Father	+			+	+	+		
Mother	+	+	+ (uterus)	+	+	+		
Donor (oocyte)	+	+	+	+			+	
Lesbian partner (oocyte)	+	+		+	+		+	
Donor (spermatozoon)	+		+	+			+	
Carrier (gestational)			+				+	
Cytoplasmic donor		+	+				+	—/+
Spindle transfer		+	+	+			+	+
Cross-generational egg donor	+	+	+	+	?		+	
Cross-generational sperm donor	+		+	+	?		+	

and assisted procreation. There are a number of assisted reproduction treatments, including gestational surrogacy, which involve three persons, but are agreed to result in genetically bi-parental offspring. During cytoplasmic donation, the donor is not just assisting the embryonic development process but also donating mtDNA. Gametes from three persons were involved in conception of the babies from cytoplasmic transfer, hence a three-person procedure. The use of a donor oocyte's cytoplasm for injection into the maternal oocyte (without removing autologous ooplasm or nucleus) may have benefited the early stages of development, although this has not been proven. A permanent biological change in the developing fetuses from cytoplasmic transfer is highly unlikely and in any event, has not been demonstrated. A permanent sequence change of the mtDNA can also not be demonstrated without analysing children born from these procedures and matching the mtDNA sequences to those of the donors. The 11 babies that tested negative for mtDNA from the donor oocytes are genetically bi-parental even though heterologous ooplasm may have assisted the embryo to develop initially. The two positively tested babies could be considered genetically bi-parental as well, unless a difference in the hypervariable sequence can be considered a hereditary change with epigenetic consequences. This consideration remains open for debate.

Similar questions can be asked when performing spindle transfer from the maternal egg to an enucleated donor egg to prevent inheritance of mitochondrial disease (**Table 1**). The mtDNA contributions to parenthood in this intervention are greater than from the donor in cytoplasmic transfer as the heritable change is presumably always permanent. Nearly all of the mtDNA in the resulting embryo will have the sequence of the donor ooplasm's mitochondria (Craven et al., 2010; Tachibana et al., 2009). In some germinal vesicle transfer experiments between different mouse strains, the interaction between nucleus and mitochondria was altered permanently as a consequence (Cheng et al., 2009). Such a complicated manipulative process at this vulnerable stage of oocyte development is not currently contemplated in clinically assisted reproduction. In contrast, generation of heteroplasmy at the mature oocyte and zygote stages in both cow and mouse after synchronous transfer of ooplasm between closely related animals from similar breeds or strains has not resulted in long-term measurable consequences for development (Cheng et al., 2009; Chiaratti et al., 2011). However, the transfer of cytoplasm between evolutionarily dissimilar mouse strains has resulted in heteroplasmic conditions which have led to physiological impairment (Acton et al., 2007). Clinical spindle transfer for the permanent treatment of mitochondrial disease would presumably normalize mitochondrial function and energy metabolism; however, unexpected epigenetic changes may occur similar to those seen in animal studies.

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