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Science Briefing

Mitochondrial Donation: Serious Concerns for Science, Safety and Ethics

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Mitochondrial Donation: Serious Concerns for Science, Safety and Ethics

*Maureen L. Condic, Ph.D.**

The governments of the United Kingdom and the United States are currently considering regulations that would allow an artificial reproductive technology that creates so-called "three-parent" embryos via a process known as mitochondrial replacement therapy or mitochondrial donation. Three separate procedures have been proposed, yet in all three cases, a human being is produced using essential components from three parents: nuclear DNA from the two intended parents and egg cytoplasm from a donor.[1]

Proponents of conducting human experiments to generate "three parent embryos"[2] initially cast this procedure as a beneficent therapeutic approach to treat women with mitochondrial disease and allow them to bear healthy children. But close on the heels of the approval of the techniques by the British House of Commons, one of its pioneers has reportedly filed for permission from the United States FDA to use these techniques as a fertility treatment for women who find it difficult to get pregnant because of their age.[3]

Whatever the purported justifications, public officials considering the legalization of these methods of genetic engineering should clearly understand the science showing that all three procedures involve genetic modification, i.e. "modifications to the subject's germ line genetic identity."[4]; all three procedures carry significant risks to the children intended to be born; and two of the procedures involve a eugenic form of reproductive cloning, in which a human being with a medical condition is killed and his or her parts are used to create a new human being with an intended improved biological state.

The scientific, safety, and ethical questions raised by this practice are profound and are in need of deeper consideration. Section I of this paper will set forth the mechanics of the three methods proposed for mitochondrial donation (Maternal Spindle Transfer, Pro-nuclear Transfer and Embryo Cell Nuclear Transfer), all of which pose unacceptable risks for the intended offspring and constitute unethical experimentation on human beings. Section II will set forth the serious

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[1] This text is a modified and expanded version of an article that first appeared in Public Discourse: Condic, M.L. (2014). We Are Not Just Our DNA: The Ethical Dangers of Three-Parent Embryos. Public Discourse (available: http://www.thepublicdiscourse.com/2014/03/12897/).

[2] Mitochondrial replacement therapy in reproductive medicine. Wolf DP, Mitalipov N, Mitalipov S. Trends Mol Med. 2014 Dec 10. pii: S1471-4914(14)00215-9.

[3] Connor, S. (8 Feb. 2015). Scientist who pioneered "three-parent" embryo technique now wants to offer it to older women trying for a baby. The Independent (available: http://www.independent.co.uk/news/science/threeparent-embryos-an-ivf-revolution-or-a-slippery-slope-to-designer-babies-10031477.html).

[4] Article 9(6) of the EU Clinical Trial Directives states that "No gene therapy trials may be carried out which result in modifications to the subject's germ line genetic identity."

health risks to the children intended to be born, including increased incidence of birth defects, cancer and metabolic diseases. Section III will raise serious ethical concerns that have not been adequately addressed.

I. Three proposed methods of treating mitochondrial disease

Mitochondria are small structures within cells that supply the energy required for life. They are unusual in that they have their own DNA that produces some of the molecules required for energy metabolism. And when this mitochondrial DNA (mtDNA) has a mutation, medical conditions affecting energy production (i.e. metabolic disease) can result. A curiosity of mammalian biology is that unlike nuclear DNA (nDNA) that is inherited equally from both parents, all of the mitochondria are inherited from the mother. So, if the mother carries a mutation in her own mtDNA, her children will have the same mutation (Figure 1). It is in the context of this biology that three methods have been proposed to avoid the birth of children with certain mitochondria based diseases or health conditions.

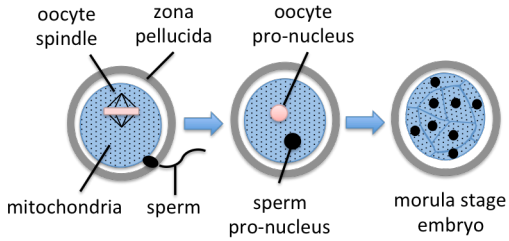

Figure 1: Normal human development. The nuclear material (DNA and proteins) of the egg is contained in a structure call the **oocyte spindle**. The egg cytoplasm contains small organelles known as **mitochondria** (small dots). All of the mitochondria of the embryo come from the oocyte. The oocyte is surrounded by a protective coat known as the **zona pellucida**. At fertilization, the one cell embryo or zygote is formed. Within a few hours, the DNA derived from the oocyte and the sperm are enclosed in **pronuclei**, which will combine to form the embryonic nucleus. By three days post sperm-egg fusion, the embryo has reached the **morula** stage of development, and each cell (blue borderlines) has mitochondria from the mother's egg and a nucleus (large dot) containing DNA from both

1. Maternal Spindle Transfer

The procedures under consideration fall into three general classes. The first, known as Maternal Spindle Transfer (Figure 2), would use an egg from the mother with the mitochondrial disease

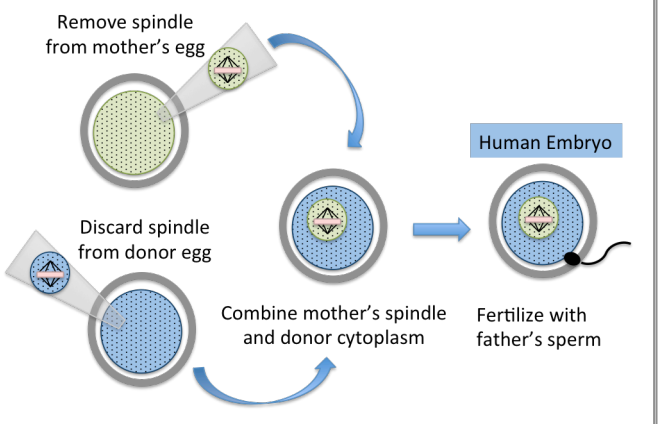

Figure 2: Maternal Spindle transfer. The spindle containing the oocyte DNA is discarded from a donor egg with healthy mitochondria (**blue**). The spindle from an egg of the intended mother is also removed, along with a small number of disease-causing mitochondria (**green**). The spindle from the intended mother is recombined with the enucleated donor egg, and the resulting "reconstituted" oocyte is fertilized by sperm of the intended father to produce a one-cell **human embryo** or zygote where the nuclear DNA is derived from the intended parents and most mitochondria from the donor. Having two types of mitochondria is known as **heteroplasmy**.

and a donor egg from a woman with healthy mitochondria. The nucleus of the donor's egg would be replaced by the nucleus of the mother's egg. This would create a "hybrid" egg, with the nDNA from the mother and the cytoplasmic elements of the egg cell (including healthy mitochondria) from the donor. This hybrid egg would then be fertilized by the father's sperm to create a "three-parent" human embryo: nDNA from the mother, nDNA from the father, and non-nuclear components (including mtDNA) from the donor.

Maternal Spindle Transfer is a risky experiment with an uncertain outcome. If either the mother's nucleus or the donor's egg is damaged, the embryo resulting from fertilization of this experimentally reconstructed cell will develop abnormally o r die. Indeed, the success rate for this procedure in animals very low (see below; *Serious Medical Risks*), raising the important question of how many human "casualties" we are willing accept in the hope of producing one human who survives and is free of disease.

Despite this serious safety concern, Maternal Spindle Transfer is essentially a manipulation of human *cells*, not human n *beings*. Consequently, it is the least ethically problematic of the three proposals. By contrast, the two other procedures under consideration ("Pro-Nuclear Transfer" and "Embryo Cell Nuclear Transfer") involve the direct destruction of at least one embryo and the subsequent use of its parts to create a new, cloned embryo with superior biology. Given the uncontested scientific evidence that human life begins at the moment of sperm-egg fusion,[5] Pro-Nuclear Transfer and Embryo Cell Nuclear Transfer are not only destructive human experimentation, they are also both forms of eugenic, reproductive cloning, conducted at a very early stage of the human life span.

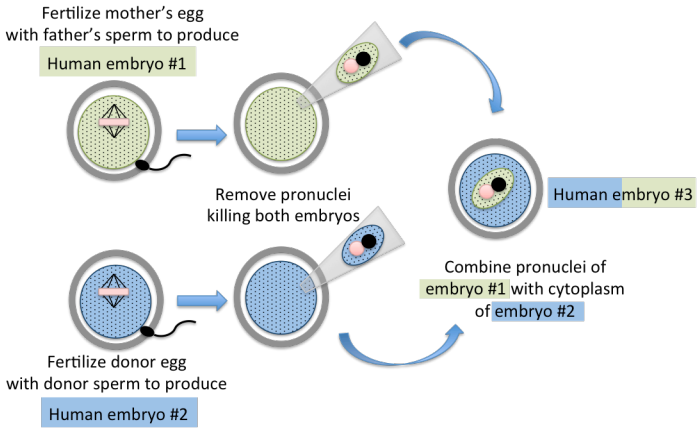

Figure 3: Pro-nuclear transfer. **Human embryo #1** is produced by fertilizing an egg cell from the intended mother by sperm from the intended father. This embryo has disease-causing mitochondria (**green**). **Human embryo #2** is produced by fertilizing a donor egg with donor sperm. This embryo has healthy mitochondria (**blue**). The pronuclei are removed from both embryos, killing them both. Then **human embryo #3** is produced by combining the pronuclei from embryo #1 with the cytoplasm of embryo #2. Embryo #3 has nuclear DNA derived from the intended parents and heteroplasmy.

2. Pro-Nuclear Transfer

In Pro-Nuclear Transfer (Figure 3), a single-cell embryo is created using sperm and egg from the intended

[5] Condic, M.L. (2008). When does human life begin? A scientific perspective. Westchester Institute White Paper 1, 1-18. Westchester Institute for Ethics & the Human Person, Thornwood, NY. (available at: http://www.bdfund.org/whitepapers). [Reprinted in: Natl Cathol Bioeth Quart. 9, 127-208.]; Condic, M.L. (2014). When does human life begin? The scientific evidence and terminology revisited. Journal of Law and Public Policy. Vol. 8 No. 1: 44-81.; Condic, M.L. (2014). Totipotency: What it is and what it is not. Stem Cells and Development 23, 796-812.

mother and father. This embryo has disease-causing mitochondria from the mother. At the same time, a second embryo is created using a donor egg with healthy mitochondria and donor sperm. Based on a large body of uncontested scientific evidence, these one-cell human embryos are clearly human beings at the earliest stage of the natural lifespan.[6]

In the next step of this procedure, the "pro-nuclei" (nuclei derived from the sperm and egg, containing the embryo's nDNA; see Figure 1) are removed from both embryos—killing them both. Then a *new* embryo is produced by transferring the pronuclei from the intended parents to the healthy cytoplasm derived from the "host" embryo. This is a form of destructive human cloning; i.e. the nuclear DNA of one human being is used to create a genetic copy or "clone" of that individual by transfer to egg-derived cytoplasm from a host embryo, killing the original embryo and the host embryo in the process (see "Serious Ethical Concerns").

In this procedure, as in Maternal Spindle Transfer, there is significant risk of damaging manipulated cells, causing development of the resulting, cloned embryo to be abnormal. In addition, two embryos are created and then destroyed in the process of producing the third, cloned embryo. Finally, this procedure involves the intentional creation of a "defective" human being ("Human Embryo #1"; Figure 3) who is then destroyed so that parts of the body (the pro-nuclei) can be used to clone a new human being, who is viewed as biologically superior. Pro-Nuclear Transfer is not a medical therapy designed to cure an individual disease. It is a manipulation that *destroys* both the individual with disease and a second healthy individual who is unrelated to the parents in order to cobble together a *superior* individual from the body parts. It is *destructive, eugenic, reproductive* human cloning.

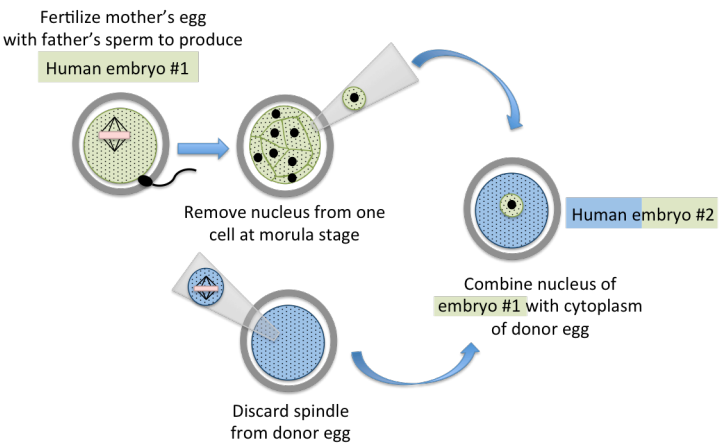

Figure 4: Embryo Cell Nuclear Transfer. **Human embryo #1** is produced by fertilizing an egg cell from the intended mother by sperm from the intended father. This embryo has disease-causing mitochondria (**green).** At the morula stage, the nucleus of one of its cells is removed, along with a small number of disease-causing mitochondria. After this, embryo #1 is destroyed. The spindle containing the oocyte DNA is discarded from a donor egg with healthy mitochondria (**blue**). **Human embryo #2** is produced by combining the nucleus from one cell of embryo #1 with the cytoplasm of the donor oocyte. This embryo has nDNA derived from the intended parents and heteroplasmy.

3. Embryo Cell Nuclear Transfer

Embryo Cell Nuclear Transfer (Figure 4) is similar to Pro-Nuclear Transfer, except that only one embryo is produced from the sperm and egg of the intended parents. This embryo is allowed to develop for a day or two and then a nucleus from one of its cells is used to produce a new, cloned embryo by transferring the nucleus to a donor

[6] ibid

egg cell with healthy mitochondria that has had its own nucleus removed. The original embryo with the mitochondrial disease is then destroyed.

In this case, as in the two procedures above, there is a significant risk of damaging the egg cell or the transferred nucleus, resulting in abnormal or failed development of the resulting cloned embryo. And Embryo Cell Nuclear Transfer is also a form of eugenic, reproductive human cloning where a "defective" human being is destroyed to obtain desirable parts (in this case, unique nDNA derived from both parents) to construct a superior human being.

II. Serious Medical Risks

All three of the proposed procedures are highly likely to be unsafe for the resulting children, setting aside the requirement that embryos are deliberately destroyed by the procedures themselves. Surprisingly, the large number of serious medical risks associated with the proposed "therapeutic" techniques have not been seriously discussed. ***Importantly, any one of the concerns listed below is sufficient to justify a ban on the proposed procedures as unsafe for use in human subjects.***

1. **Risks associated with damage to the nucleus or the egg**: As noted above, all of the proposed techniques carry an inherent and significant risk of damage to the nucleus or to the egg cell. While four live-born monkeys have been produced using this technique, this "success" does not reflect the large number of embryos that were so damaged or defective, they were unable to progress to maturity. Indeed, in two separate papers using Maternal Spindle Transfer, only 46 out of 85 monkey embryos produced[7] or 19 out of 60 human embryos produced[8] were able to progress even to the blastocyst stage (approximately day 5-7 of development). In the first study, 15 monkey embryos were transferred to surrogate mothers, yet only four pregnancies resulted. Therefore the "healthy" offspring produced by this technique represent a small fraction of all the embryos that were originally generated, *indicating that this procedure was lethal for the great majority of the embryos it produced*. Moreover, although these four animals appear normal,[9] this is hardly a sufficient number to conclude the procedure is safe, even for the small number of individuals that survive the procedure itself.

2. **Risks associated with nuclear transfer (cloning):** It is unambiguously established by a large number of independent studies that cloned animals are not healthy. Despite decades of experience, cloning is an inherently risky procedure, with success rates typically in the range of 0.1-1%; i.e. 99.9-99% of all cloned animals are so abnormal they do not survive to live birth.[10]

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[7] Mitochondrial gene replacement in primate offspring and embryonic stem cells. Tachibana M, Sparman M, Sritanaudomchai H, Ma H, Clepper L, Woodward J, Li Y, Ramsey C, Kolotushkina O, Mitalipov S. Nature. 2009 Sep 17;461(7262):367-72.

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[9] Ibid.

[10] Cloning from stem cells: different lineages, different species, same story. Oback B. Reprod Fertil Dev. 2009;21(1):83-94. Climbing Mount Efficiency--small steps, not giant leaps towards higher cloning success in farm

Even the rare individuals who do survive exhibit serious disregulation of multiple genes that result in significant medical problems.[11] These abnormalities do not arise as a consequence of subtle, gestational irregularities, but are seen from the very beginning. Direct examination of embryonic stem cell lines derived from cloned embryos at approximately 5-7 days of development also shows that, like the embryos they are derived from, cloned ESCs are also clearly "abnormal" when compared to ESCs derived from fertilization.[12] In light of the strong evidence that cloning produces abnormal embryos, Pro-nuclear transfer and Embryo Cell Nuclear Transfer (both forms of reproductive cloning) are not safe for use in humans.

3. **Risks associated with producing babies in vitro:** While most human infants born as a result of assisted reproductive technologies (ART) are healthy by most measures,[13] a growing body of data indicates that children produced in the laboratory are at significantly greater risk for a wide range of medical issues, including neurological disorders,[14] cancer[15], congenital

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abnormalities,[16] and imprinting disorders (Beckwith-Wiedemann syndrome, Silver-Russell syndrome, Angelman syndrome and others).[17] Similar results are well established in multiple species of animals produced by ART[18]. Moreover, ART-produced individuals are at significantly higher risk for premature birth and low birth weight, with all of the many long-term consequences associated with these events.[19] While some argue that these medical problems are caused by the "subfertility" of the parents, children conceived by ART are at greater risk compared to children of similarly "subfertile" parents who conceived naturally.[20] In many cases,

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we are only now appreciating the complications of ART, as individuals produced in the laboratory are reaching adulthood.[21] Permitting even greater risk to ART produced children by allowing grossly invasive procedures such as cloning or Maternal Spindle transfer to also be performed is medically irresponsible.

4. **Risks associated with heteroplasmy:** In all three of the procedures outlined above, there is a significant risk of transferring some of the disease-carrying mitochondria from the mother's egg to the baby, resulting in a mix of healthy, and disease-carrying mitochondria; a condition known a "heteroplasmy" (See Figures 2-4). In this case, heteroplasmy could cause reappearance of the disease in the offspring of any woman produced by the "three-parent" approach, due to mitochondrial "founder effects" in oogenesis.[22] Even a few "bad" mitochondria can become the dominant type in any one egg, causing the mitochondrial disease to recur in any child produced from that egg.

5. **Risks associated with mtDNA-nDNA incompatibility:** All of the proposed procedures are forms of "germ-line engineering" that alter the genetic makeup of future generations in a permanent way. We know that in nature, mtDNA and nDNA "co-evolve" to work with each other in an efficient manner.[23] In some species,[24] incompatibility between the mitochondrial and nuclear genome significantly compromises the health of the individual. And there is growing evidence that such "incompatibility" also contributes to human pathology.[25] All of the proposed methods of "treating" mitochondrial disease introduce a permanent and unnatural mismatch

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between the nuclear and the mitochondrial genome that will be inherited by all subsequent generations. Producing such genetically modified (GM) humans constitutes unethical, destructive experimentation on humans, with no guarantee of a safe outcome for either the "patient" (the cloned embryo produced) or any of the offspring of that patient. This is an unwarranted approach that puts future generations at grave risk of unforeseen consequences, in addition to the clearly foreseen destruction of a class of "defective" humans in the hope of manufacturing "superior" offspring.

III. Ethical Concerns

A. Genetic engineering, not "therapy"

Surprisingly, many people do not see an obvious ethical problem with these proposals. A medical colleague of mine recently opined, "If you take the newly formed pronucleus and put it in a different 'body' (i.e., another woman's egg), are you really destroying that embryo? The individual would still develop with almost all the same genetic traits, and would potentially survive longer if the therapy worked."

Yet the view that transfer of an embryo's nucleus is merely a "therapeutic" approach for treatment of disease is false. The embryo produced by this procedure is not just the original child of the parents, moved to a new cytoplasmic "environment." This would only be true if a human being were nothing more than his or her nDNA, which is clearly not the case. While our unique DNA clearly determines many aspects of our individual characteristics, we are also critically affected by the specific, non-genetic composition of the egg that produced us. As explained in detail in elsewhere,[26] many aspects of embryonic development, and therefore many aspects of the unique individual we end up being, depend on non-genetic components derived from the cytoplasm of the egg.

The importance of non-genetic factors in determining the unique character of a human individual is very clearly illustrated by "maternal effect mutations."[27] These mutations have no effect on the development or function of the mother, but specifically disrupt development of embryos derived from her eggs. The embryo may not even have the "bad" gene (only half of the mother's genome is passed on to any one child), but embryonic development can still be profoundly affected by the molecules present in the egg itself. Many key developmental factors work like this—in both positive and negative ways. Therefore, all three of the procedures described above would indeed generate "three-parent embryos," whose unique traits and human identity would reflect the genetic contributions of both the genetic mother and father as well as the critical, non-genetic contributions made by the egg donor.

B. Other Serious Ethical Concerns

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In addition to the concerns raised by genetic engineering and the unnatural production of genetically modified human beings with three biological parents, a number of other serious ethical concerns raised by the proposed techniques are noted briefly below.

1. Ethical issues raised by intentional production and subsequent destruction of large numbers of human beings in order to use their body parts for the production of a third, biologically superior human being; i.e. *destructive eugenic cloning*.
2. Ethical issues raised by *human reproductive cloning*.
3. Ethical issues raised by generation of a *large number of damaged embryos as "casualties"* in an attempt to produce one individual who is free from disease.
4. Ethical issues raised by the *greatly increased medical risks* for the children who survive this procedure.
5. Ethical issues raised by permanent, germ-line engineering of human beings (*GM-humans*), with no way of predicting the long-term outcomes for the individuals produced in this manner.

C. Conclusions

Producing three-parent embryos is a form of eugenic, human experimentation. In the most extreme forms (Pro-nuclear Transfer and Embryo Cell Nuclear Transfer), this approach constitutes destructive human cloning, not for research or advancement of knowledge, but rather with the express intention of producing cloned human babies. The risk to the babies produced cannot be predicted and is likely to be significant. This approach will, for the first time, produce genetically modified human beings (GM-humans). It is unsafe, unethical and irresponsible to allow such experimentation on innocent human subjects who will be forced to live with whatever unforeseen consequences may result.