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SHOULD THE U.S. APPROVE MITOCHONDRIAL REPLACEMENT THERAPY?

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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ABSTRACT

The overall goal of this project was to document and evaluate the new technology of mitochondrial replacement therapy (MRT), and to assess its technical, ethical, and legal problems to help determine whether MRT should be approved in the U.S. We performed a review of the current research literature and conducted interviews with academic researchers, *in vitro* fertility experts, and bioethicists. Based on the research performed for this project, our team's overall recommendation is that the FDA approve MRT initially for a small number of patients, and follow their offspring's progress closely for a few years before allowing the procedure to be done on a large scale. We recommend the FDA approve MRT only for treating mitochondrial disease, and recommend assigning parental rights only to the two nuclear donors. In medical research, animal models are useful but imperfect, and *in vitro* cell studies cannot provide information on long-term side-effects, so sometimes we just need to move forward with closely monitored human experiments.

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AUTHORSHIP

Author	Areas Covered
Daniela Barbery	<p>Background on mitochondrial diseases. Which genes are mutated in mtDNA diseases? Which diseases will best be served by MRT?</p>
Emily Caron	<p>Background on <i>In Vitro</i> Fertilization (IVF). Problems encountered with IVF technology. Will any IVF problems also apply to MRT?</p>
Benjamin Grondin	<p>MRT technique. How was MRT developed? What are MRT potentials and problems? Can MRT also treat the patient, not just the offspring?</p>
Daniel Eckler	<p>IVF and MRT Ethics. Do MRT problems outweigh the benefits? Will MRT be expensive?</p>
Maureen Hester	<p>IVF and MRT Legalities. How was MRT approved in England? What steps remain in the U.S. for approving MRT? Is MRT tourism likely?</p>

PROJECT GOALS

The overall goal of this project is to document and evaluate the new technology of MRT, and to assess its technical, ethical, and legal problems to help determine whether MRT should be approved in the U.S.

The specific objectives are to:

- 1 **Develop** a comprehensive assessment of the scientific experiments that led to the development of mitochondrial replacement therapy, and discuss the technique's potential applications.
- 2 **Characterize** what key scientific and IVF stakeholders believe are the strengths and weaknesses of this technology, and their ethical and legal concerns.
- 3 **Evaluate** all of the obtained evidence and prioritize the remaining problems.
- 4 **Recommend** potential solutions to any remaining problems for the approval of MRT in the U.S.

EXECUTIVE SUMMARY

Mitochondria are DNA-containing organelles present in the cytoplasm of eukaryotic cells. They primarily function to provide the cell's energy. Mitochondria are originally thought to have been free-living *Rickettsia* proteobacteria engulfed by a larger cell, so they contain their own DNA (mitochondrial DNA, mtDNA). Over time, most of the mitochondrial genes moved into the nuclear DNA, so most mitochondrial proteins today are actually encoded by nuclear DNA, are synthesized from mRNA in the cytoplasm, and are imported into the mitochondria. Mitochondria are maternally inherited. They are passed to the offspring through the egg's cytoplasm, deposited there during egg formation (oogenesis). During fertilization, the male mitochondria either do not enter the egg or are quickly degraded upon entrance, so male mitochondria normally do not become part of the embryo.

Mitochondrial diseases are caused by mutations in mitochondrial DNA genes, or by mutations in nuclear genes encoding proteins that function within mitochondria. Because mitochondria are found in almost all cells in the body and they are extremely important in energy production, mitochondrial diseases can affect any tissue in the body. However, the diseases mostly manifest in tissues that rely strongly on energy production, such as brain, heart, muscle, pancreas, and kidney. Mitochondrial diseases can affect a specific organ, or can affect a group of organs. There is no single age of onset.

This year, after much debate, England became the first country in the world to approve the use of a new procedure for potentially preventing the transfer of diseased mitochondria to offspring during fertilization. The technique is termed mitochondrial replacement therapy (MRT). MRT is a type of *in vitro* fertilization (IVF) procedure, and begins with a mother with diseased mitochondria providing her eggs for IVF. During MRT, the nucleus of one of these eggs is removed and injected into an enucleated donor egg containing healthy mitochondria. The injected egg is then fertilized by the father's sperm. The embryo is cultured *in vitro* for 2-5 days, and is then implanted into the mother's uterus to hopefully create healthy offspring. Because MRT is a type of *in vitro* fertilization (IVF), it comes with similar technical, ethical, and legal problems; but MRT also has problems of its own.

The overall goal of this IQP project was to document and evaluate the new technology of MRT, and to assess its technical, ethical, and legal problems to help determine whether MRT should be approved in the U.S. The specific objectives were to: 1) Develop a comprehensive assessment of the scientific experiments that led to the development of mitochondrial replacement therapy, and discuss the technique's potential applications. 2) Characterize what key scientific stakeholders believe are the strengths and weaknesses of this technology, and their ethical and legal concerns. 3) Evaluate all of the obtained evidence and prioritize the remaining problems. 4) Recommend potential solutions to any remaining problems for the approval of MRT in the U.S.

To accomplish objective-1, we performed a review of the current literature, including reputable academic journal articles, relevant books, scholarly websites, and other pertinent materials. To accomplish objective-2, we conducted a set of interviews with various academic

researchers. The interviewees included individuals working with mitochondrial disease patients, individuals performing IVF or MRT procedures, bioethics experts, and MRT legal experts. The purpose of the interviews was to determine the interviewees full range of opinions on MRT, and to solicit their help gauging the strengths and weaknesses of this new technology. After performing the Literature Review and interviews, the group synthesized all of the information collected to ascertain the strength of the evidence for and against MRT, and created recommendations for approval of MRT in the U.S. and for further research.

Based on the research performed for this project, our team made several conclusions and recommendations (also discussed in the Results and Conclusions sections), but are briefly summarized here.

Which Mitochondrial Disease?

With respect to which specific mitochondrial disease might best be served by MRT, our interviews with several mitochondrial disease experts indicated that all types of mitochondrial diseases would be treatable by MRT, not just one type, so long as the mutation lies in mtDNA not in nuclear genes encoding mitochondrial proteins (which would remain unaffected by MRT). We agree with the interviewees who pointed out that if approved by the FDA in the U.S., decisions whether to use MRT for a patient would be made on a case-by-case basis between the patient and her physician, and would likely include factors such as her overall health, age, and extent of disease symptoms. Some interviewees reminded us that MRT affects the offspring not the original patient, but those interview responses occurred before the late summer 2015 publication of a technique deriving stem cells from MRT patients that might be used in the future for treating the patients themselves ([Ma et al., 2015](#)).

Heteroplasmy

One of the most important concerns identified in our MRT research was the worry by some individuals that heteroplasmy would negatively affect MRT offspring. Heteroplasmy is the existence of two different types of mitochondria inside one cell. During MRT, this can potentially occur with the carry-over of small amounts of diseased mitochondria with the nuclear sample during its microinjection into the healthy egg.

The concerns for heteroplasmy originate from the fact that the nucleus and mitochondria must constantly interact to make a functional mitochondria, so changing the type of mitochondria in a cell might create interaction problems. Although mitochondria may at one time have been free-living ancient *Rickettsia* proteobacteria with their own complete DNA genome, over time most of those genes moved into the nucleus, so that today's mitochondria now contain only about 37 genes, with the majority of mitochondrial proteins actually being encoded by nuclear DNA. The nuclear-encoded mitochondrial proteins are synthesized in the cytoplasm and are then imported into the mitochondria. So, the nucleus and mitochondria must constantly interact, and the proteins encoded by nuclear DNA must be compatible with (provide proteins that fully function) inside the cell's mitochondria. Some scientists worry that "problems could arise if mitochondrial and nuclear DNA from different women prove to be incompatible", and have cited

dozens of experiments in mice, fruit flies, and other animals where mixing nuclear and mitochondrial DNA from individuals with different genetic backgrounds sometimes led to problems such as reduced growth, early death, accelerated ageing or reduced reproductive ability.

Adding fire to the heteroplasmy worry is a set of children born in New Jersey in 1997 and 1998 that inadvertently became the world's first genetically modified offspring. Dr. Jacques Cohen, then at the Institute for Reproductive Medicine and Science of Saint Barnabas (Livingston, NJ) performed ooplasmic (egg cytoplasm) transplantation experiments to attempt to treat infertility in seven couples with multiple implantation failures. Dr. Cohen wondered what would happen if he added a little cytoplasm from a healthy woman's egg into the eggs of the infertile women. They injected less than one picoliter (10^{-12} liter) of another woman's egg cytoplasm into 33 previously infertile eggs (and the injected cytoplasm contained mitochondria from the donor). Overall, 17 babies were born from this study and a later study. In 2000 and 2001, scientists at the same location in New Jersey used mtDNA fingerprinting to show that tissues isolated from the children contained mitochondrial heteroplasmy, proving that both types of mitochondria (original and donor) survived in the baby's cells, and providing the first hard evidence of human "germline" genetic modification. The children's health is only now being monitored for long-term effects. They are now in their late teens and have not gone through middle-age, a time that animal experiments show might be important. Animal experiments show that MRT mice develop hypertension and obesity in middle age, and show impaired cognition. One of the ooplasm-injection children developed autism, and two fetuses developed Turner Syndrome (one miscarried and one aborted).

While some scientists think heteroplasmy might be a problem, other scientists argue it will not be a problem during MRT because these transfer experiments merely recapitulate what is happening everyday worldwide without any known adverse effects when a male offspring is born (Chinnery et al., 2014). Following fertilization, the male embryo's new nuclear DNA (recombined from the mother and father's DNA) now exists in a new tandem with the mother's mitochondrial DNA, and healthy male boys are born worldwide daily. Moreover, the sequence variation between different mitochondrial haplotypes in the human population is actually very small, translating to just a few amino acid substitutions (Mitalipov and Wolf, 2014). So, these scientists argue that mitochondria really do not differ that much from each other, and mitochondrial heteroplasmy should not be an issue. One key study cited in our Lit Review performed MRT on human eggs and showed no obvious developmental problems grown to the blastocyst stage. Another key study created several MRT monkeys, and their MRT embryos showed no obvious morphological or developmental timing alterations relative to normal WT embryos, and the born MRT offspring so far appear to be normal. One scientist that we interviewed cited a recent study of more than 150 pathological DNA point mutations indicating that the probability of disease symptoms (either mild or severe) is very low for mutation loads below 18%. Another interviewee thought that a mere 2% carryover of diseased mitochondria would be asymptomatic in MRT offspring because many "normal" people naturally have this extent of heteroplasmy without even knowing it or being bothered by it.

With respect to heteroplasmy, we conclude that the mere 1-2% heteroplasmy resulting from MRT should not be a problem and should provide no negative symptoms. The 1-2% levels are far less than the level of heteroplasmy observed in the cited animal experiments where it was determined to be a problem, and many individuals naturally exist with this level of heteroplasmy

with no problems. And in some cases, any observed heteroplasmy actually decreases further with increased cell division of the embryo.

Problems with IVF

In vitro fertilization (IVF) is a type of assisted reproductive technology (ART) in which an egg is fertilized by sperm outside the body. The process involves several key steps, each of which has potential problems: 1) monitoring and stimulating a woman's ovulatory process, 2) surgically removing an ovum or ova (egg or eggs) from her ovaries, 3) mixing the egg with sperm (or injecting sperm into the egg) to allow fertilization, 4) culturing the fertilized zygote for 2-6 days in a growth medium to create a cleavage-stage embryo or a blastocyst, and 5) implanting the embryo into the woman's uterus (or that of a surrogate) to hopefully establish a pregnancy. Several variations of IVF exist, including the use of a pregnancy surrogate, the use of donated eggs, or the use of donated sperm. IVF is frequently used to overcome female infertility resulting from blockage of the fallopian tubes, where the eggs no longer successfully move to the oviducts to become fertilized. IVF can also be used to assist with male infertility, especially in cases of low sperm quality or quantity. *In vitro* fertilization is now a well-established technology with a variety of applications in basic and applied sciences. IVF treatments can now routinely provide higher quantities of good quality eggs, and higher percent fertilization success.

However, IVF technology also has several problems, some of which may apply to MRT. These problems include: 1) side-effects of the hormones used to induce egg development, some of which have been shown to cause ovarian hyper-stimulation syndrome (OHSS) and a decrease in live-birth rates. 2) Age: IVF success rates strongly decline with increasing age of the patient, so the age of the MRT patient likely will be important. 3) Stress: Stress decreases IVF success rates, so stress must be minimized during MRT. 4) Cost: IVF is a very expensive procedure. In the U.S., IVF is not covered by most medical insurance plans. Will MRT (involving three people, not two) cost even more than IVF? Will medical insurers likely treat MRT (and its potential to eliminate a fatal disease) differently than IVF (where the patient is looking to have a baby)? 5) Equipment: Some studies suggest that doctor training and use of specific types of equipment can significantly improve IVF success rates. This likely will strongly apply to MRT.

The topic of IVF-induced birth defects is one of the most controversial topics in the IVF field. Although some studies have shown an increased risk of specific disorders associated with IVF, other studies argue the risks are slight and may instead be associated with the underlying infertility problems not the IVF technique. Some of these IVF problems were followed up in interviews to use experts in the IVF field to help prioritize which topics are likely to be most problematic for MRT. Some studies performed in our review of the literature found a significantly increased risk of pre-term birth (<37 weeks old) associated with IVF when implanting blastocyst embryos (5-6 days old) into the uterus instead of younger cleavage-stage embryos (1-3 days old). Our interview with the senior author of that study indicated the increased time of culture of the embryo in medium to the blastocyst stage may cause problems not seen if the embryo is transplanted earlier, so perhaps that earlier implantation procedure should be implemented during MRT. Other scientists have developed new morphokinetic procedures for using time-lapse video microscopy to help determine which embryos are healthiest for transfer based on a combination of the IVF embryo's appearance (morphology) and the kinetics of key cellular processes that affect morphology. So, perhaps this new procedure

could be applied to MRT to improve its success rates. When interviewed on this subject, the author of the study agreed this technology could in theory be applied to MRT. Some studies have shown that using a trigger shot of the hormone GnRH α during IVF procedures instead of the traditional hCG helps eliminate ovarian hyper-stimulation syndrome (OHSS). Our interview with the corresponding author of that study indicated he agreed that the technique would also apply to MRT, so we recommend using GnRH α as the trigger. With respect to age, our review of the literature identified several studies showing that an increased age of the IVF patient strongly negatively correlates with IVF success rates. Our interview with the corresponding author of one of those studies validated our view that elevated age would also negatively impact MRT, so likely doctors will strongly consider the age of the mother (along with other factors such as overall health and symptoms) before performing MRT.

Although some of the IVF findings will likely apply to MRT (increased patient age leading to decreasing MRT success, the use of GnRH eliminating ovarian hyper-stimulation syndrome, the implantation of cleavage stage embryos not blastocysts to increase success, and the use of the new morphokinetic procedure for monitoring embryos), not all IVF procedures will automatically apply to MRT. One interviewee whose own research showed an increase in birth defects associated with IVF, cautions that IVF findings may not always pertain to MRT because the two patient populations are very different. IVF couples have underlying fertility problems which affect success outcomes, while MRT would be performed on patients suffering from a very different type of disease. Another key interviewee pointed out that ART is a rapidly developing field, and with epidemiological research it takes a few years to collect a sufficient amount of data, so at this time it is very difficult to say whether MRT technology would carry similar risks, but in any case it will be important to monitor MRT offspring long-term to determine potential effects not only from the beginning but also after middle age.

MRT and Modification of the Germline

With respect to bioethics, the main ethical reason MRT is controversial is it modifies the DNA of the offspring such that it is passed on to future generations. And this modification is done without the individual's (offspring) consent. Previous types of human gene therapy experiments corrected a specific gene for one individual's DNA to treat a specific genetic disease, but in those cases, the risks were assumed only by a consenting individual (usually a patient who had already exhausted all other forms of treatment for their fatal or debilitating disease). But with MRT, according to Marcy Darnovsky, Executive Director of the Center for Genetics and Society, "Unlike experimental gene therapies where risks are taken by consenting individuals, [MRT] turns children into our biological experiments, and forever alters the human germline in unknowable ways. There is no precedent for this" (Vogel, 2015). Until now, procedures that produced inheritable gene alterations have been ethically taboo (Vogel, 2014).

Some individuals worry that approving MRT will open the door for *eugenics*, the practice of using gene alterations to improve human genetic features. For example, the UK's conservative Parliament member Jacob Rees-Mogg equates MRT with cloning, and said "the technique would promote eugenics; in a country nervous about genetically modified crops, we are making the foolhardy move to genetically alter babies" (Callaway, 2014). In the U.S., in March of 2013, a group of ethicists sent a letter to *The Times*, stating that MRT would "open the

door to further genetic alterations of human beings with unforeseeable consequences” (Callaway, 2014).

But other scientists point out that MRT would only be used to treat serious mitochondrial diseases, not play with the human nuclear genome. They argue that mtDNA makes up only a tiny fraction (0.1%) of the patient’s total genome, with little influence over a human being’s *defining* traits (Callaway, 2014). MRT experts Shoukhrat Mitalipov and Don Wolf suggested that mitochondrial DNA’s contribution to the individual’s total genome “is small, constituting just 0.1% of the total DNA”, and the mitochondria gene defects to be treated with MRT “cause severely debilitating and life-threatening conditions in children, and it might be considered unethical to deny MRT gene therapies for these diseases if concerns about safety and efficacy were addressed adequately” (Mitalipov and Wolf, 2014). In addition, some individuals argue that physicians have the obligation to act on behalf of their patient’s well-being, so if a treatment exists that could potentially improve a patient’s life in a safe way, denying them such a treatment would be unjust (Klitzman et al., 2015). On a further pro-MRT note, several members of Parliament were quoted as saying that MRT is more like “changing a battery pack than about genetic modification” (Parliament.UK, 2015). The parent’s nuclear DNAs would remain unchanged. Several of our interviews verified our point that mtDNA, unlike nuclear DNA, does not specify an individual’s identity. So, does the benefit of eliminating a severe debilitating or fatal human disease override the societal objection to slightly “changing the human germ line”?

With respect to eugenics, we conclude that slightly altering a family’s mitochondrial gene line (as occurs daily during normal male births when the new nuclear DNA is matched with the mother’s mitochondria) using MRT simply does not match up to the hardship associated with mitochondria diseases in a person’s body. We agree that no evidence exists indicating that mitochondria encode proteins specifying an individual’s identity. In medical research, sometimes human studies are the only way to obtain the final information on safety, and we just have to proceed forward with a few test cases. Animal models are imperfect, and *in vitro* cell studies cannot provide information on long-term side effects, so we just need to move forward.

IVF Laws

IVF procedures have been used in humans since the birth of the first test tube baby, Louise Joy Brown, in England on July 25, 1978 (BBC News, 1978). Since that first IVF birth, the use of IVF has expanded worldwide with millions of procedures performed. Assisted Reproductive Therapy (ART) is now one of the most highly regulated of all medical practices in the U.S. (American Society...2010). It is regulated on 3 levels: state, federal, and professional self-regulation (Minieri, 2013). The state level involves the licensure and monitoring of qualified physicians who meet minimum standards for skill and education. The federal level of regulation is orchestrated by The Centers for Disease Control and Prevention (CDC), The Food and Drug Administration (FDA), and The Centers for Medicare and Medicaid Service (American Society...2010). The practices are also self-monitored and regulated by The American Society for Reproductive Medicine (ASRM) and the Society for Reproductive Technology (SRT).

Which types of IVF procedures are legal vary from country to country, often reflecting local religious influences. For example, the European Union shows a wide variety of laws

related to IVF: Croatia, the Czech Republic, Estonia, Ireland, Latvia, Luxemburg, Malta, Poland, Portugal, Romania, Slovakia, and Slovenia all fully allow access to IVF by anyone, while other European countries restrict IVF access only to specific people, such as married couples. Muslim countries in general support IVF technologies as a means to help married couples have children. Within Islam, marriage is considered a dutiful form of worship, and the second function of this union is procreation. So, the preservation of heredity is very important to Muslims. IVF is generally accepted and allowed within a marriage, so long as it helps the couple maintain their genetic lineage (Alghrani, 2013). The acceptable ways in which IVF can be practiced, however, differs between Sunni and Shite denominations. Conservative Sunni Muslim nations generally allow IVF only if performed on married couples using their own sperm and eggs, but do not allow the use of donor eggs, while Shite's allow the use of donors (Inhorn, 2011). Shiites sometimes invoke the interesting religious concept of mut'ah (temporary marriage) to allow the use of a *surrogate* donor outside the married partners, although our interview with an Islamic IVF expert indicates this interesting practice is likely in decline and is no longer needed to allow IVF surrogates.

The legal ban on IVF for some individuals in some countries has given rise to the new area of "*fertility tourism*", where an individual travels to other another country to undergo the procedure. ART regulations in different areas of the world have created "reproductive borders." Laws affect all aspects of IVF, including its high cost, long waiting lists, and limitations such as reproductive age or sexual orientation, and the country borders are being crossed to circumvent these complications and expedite the process of having a child (Bergmann, 2011). The main reason for the travel varies from country to country, but the general trend is represented, for example, by Italy where 70.6% of the respondents sought IVF in other countries due to legal bans within Italy, and 43.3% of the travelers wanted better quality care (Minieri, 2013). This same trend is reflected in other European countries. Within the U.S., no current laws prohibit international surrogacy, and an outsourcing of gestational surrogates to Indian women by U.S. citizens is becoming more commonplace for American couples hoping to have a child (Stephenson, 2009). For U.S. travelers, cost is also a major factor. The cost of using a surrogate (in 2009) in India averaged \$20,000 while the same process in the U.S. can cost \$90,000 (Stephenson, 2009). We performed an interview with a German IVF expert on fertility tourism, and he indicated that countries receiving such travelers likely will not toughen their laws on IVF because it would not be in their own interest to do so. He mentioned a legal case in Austria which resulted in the courts supporting each EU country designing and enforcing their own IVF laws; and this case also led to a significant amount of publicity for German citizens who had to travel to receive IVF treatments, and whether Germany should change its own IVF policies. He also indicated that MRT tourism would be far less common than IVF tourism due to the vastly fewer MRT patients.

England's MRT Laws

The U.K has been actively engaged in MRT debates for over 10 years now, and in 2015 became the first country to approve the procedure. As a brief history, in 1990, Britain's Parliament approved the Human Fertilization of Embryology Act of 1990 (Gov.UK, 1990) which prevented any human nuclear transfer trials (which would have included MRT if it existed then), and at the same time it established Britain's fertility regulator, the Human Fertilization and

Embryology Authority (HFEA). Among its other responsibilities, the HFEA licenses and monitors all human embryo research performed in the U.K., and these responsibilities continue to this day. In 2005, the HFEA issued a research license to the Newcastle Centre for Mitochondrial research to “investigate the feasibility of using IVF-based techniques to prevent the transmission of mitochondrial disease” ([HFEA.gov, 2005](#)). But in that year, MRT clinical trials were still prohibited by the 1990 act, so in 2008, Parliament amended the 1990 Act to allow HFEA to license and monitor MRT clinical trials, creating the Human Fertilization and Embryology Act 2008 ([Gov.UK, 2008](#)). In 2011, the HFEA convened an expert panel to review the effectiveness and safety of MRT ([HFEA Review, 2011](#)). The panel concluded in their report entitled “Scientific Review of the Safety and Efficacy of Methods to Avoid Mitochondrial Disease Through Assisted Conception” that there was no scientific evidence showing that MRT is unsafe ([HFEA Review, 2011](#)). Their updated reports in 2013 and 2014 concluded the same.

In 2012, England’s HFEA initiated a public consultation process on the social and ethical implications of MRT. The outcome, published in 2013 ([HFEA Advice to Government, 2013](#)), was a general support for permitting MRT so long as it is safe and done within a regulatory framework. On February 3, 2015, the House of Commons voted to approve (by a vote of 382 in favor and 128 against) an amendment to the earlier “Human Fertilization and Embryology Act 2008” which allowed MRT clinical trials ([Callaway, 2015](#)). The amendment then moved to the House of Lords, which voted to approve the measure on February 24, 2015 ([Vogel, 2015](#)). The 2015 approval votes in the House of Commons and House of Lords makes the U.K. the first country in the world to explicitly allow MRT for the public, and gives HFEA the authorization to begin issuing licenses to facilities to perform the technique. Researchers who wish to offer the MRT service to couples in England must first apply for and receive a license from HFEA ([Vogel, 2015](#)) and that authority will likely carefully consider applications on a case-by-case basis prior to issuing any license ([Callaway, 2015](#)).

Policy makers in the UK addressed several key points in their MRT debates that likely will be of interest to the U.S. debate. They approved MRT only in cases involving mitochondrial diseases, the technology cannot be used for women who are becoming too old to reproduce, and MRT cannot be used to create “designer babies” ([Klitzman et al., 2015](#)). In addition they stated that the donor of the healthy mitochondria will not have any parental rights, those rights will be reserved for the two nuclear donors.

U.S. MRT Debate

MRT is not approved for human use in the U.S. The U.S., unlike England, lacks a single governmental agency for regulating reproductive technologies, so jurisdiction has been claimed by the U.S. Food and Drug Administration (FDA), especially the FDA’s Office of Cellular, Tissue, and Gene Therapies of the Center for Biologics Evaluation and Research ([FDA.gov, 2001](#)). So, unless congress intervenes, the FDA currently has the power to regulate MRT as a form of gene therapy. The jurisdiction of MRT clinical trials is given to the FDA because it involves the manipulation of the germ line ([Mitochondrial...2014](#)).

Under the FDA’s regulations, MRT approval will require the performance of phase I, II, and III clinical trials using the usual Investigational New Drug (IND) application method for

approval to begin any studies. But to date, the FDA has not officially considered or approved any MRT human clinical trials. On July 2, 2013, the FDA published their Draft Guidance for Industry: Considerations for the Design of Early Phase Clinical Trials of Cellular and Gene Therapy Products ([Federal Register, 2013](#)), but that draft guidance did not mention MRT. On February 25 and 26, 2014, the FDA convened a 2-day meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee to discuss “oocyte modification in assisted reproduction for the prevention of transmission of mitochondrial disease” ([FDA.gov, 2014](#)). This committee included doctors, researchers, representatives of industry, and representatives of patient groups ([Vogel, 2014](#)). Although this 2014 meeting represented the first time the U.S. had officially discussed the MRT technique, no conclusion was made at the meeting. Several panelists went on record as saying there was “probably not enough data in animals to move to clinical trials without answering a few additional questions” ([Begley, 2014](#)). Some of the additional data the scientists wanted to see were the long-term health of monkeys conceived through MRT ([Callaway, 2015](#)). Evan Snyder, a stem cell biologist at Sanford-Burnham Medical Research Institute (La Jolla, CA), Chair of the FDA panel, said “It will probably take two to five years to fill in these [data] gaps” ([Callaway, 2015](#)). Standardization of mitochondrial manipulation techniques was also recommended. There would need to be a significant monitoring of any human clinical trials, and at least this meeting started a constructive dialogue in the ways this could and should be done.

In September 2014, the FDA commissioned an Ad Hoc Committee of the Institute of Medicine (IOM) to consider the “ethical and social policy considerations of novel techniques for the prevention of maternal transmission of mitochondrial DNA diseases” ([National Academies, 2014](#)). Two of their discussion sessions will be open to the public ([Cohen et al., 2015](#)). Until the release of IOM’s report (estimated to be about April of 2016, 19 months after the September 2014 start date), no further FDA actions on MRT are expected. In the meantime, any IND applications for MRT human clinical trials will remain on hold.

Congress may become involved in the MRT approval process in the U.S. On June 17, 2015, a draft spending bill was released in the House of Representatives that if passed would restrict any research on heritable genetic changes to human embryos. If the bill passes, it could ban the FDA from reviewing such research, and might include the technique of MRT. Representative Andy Harris (R-MD) commented on the bill saying “it would slow down the FDA’s review, allowing everyone a chance to review the ethics”, while Henry Miller, a physician and biologist at Stanford University said “it would set a new standard for congressional stupidity and inhumanity” ([News in Brief, 2015](#)). Dr. Shoukhrat Mitalipov, a biologist at the Oregon Health and Science University (Portland, OR), who has performed MRT experiments on monkeys, and who hopes to apply for FDA approval to perform human MRT, stated “Although [the US] regulatory debate is a bit behind the United Kingdom’s, the US is going down the same path” ([Callaway, 2015](#)). Australia is also considering allowing human MRT, but David Thorburn, a geneticist at the University of Melbourne said “My gut feeling is that [MRT is] unlikely to succeed [in Australia] until this has been done in practice in the U.K.” ([Callaway, 2015](#)).

Our interviews in the U.S. legal area indicated they agreed with our main finding, that the U.S. is awaiting the findings of the Institute of Medicine’s Ad Hoc Committee report on MRT, and that a recommendation of approval of that committee would be the next step for approval by the FDA. Our interviews indicated the U.S. need not pass any new legislation to move forward

with MRT (just FDA approval), unless congress intervenes to pass legislation banning the FDA from reviewing the procedure. Our interviewees agreed with our statements that eugenic applications of MRT are “impossible” given the role of mitochondrial DNA, but if it is deemed important, the FDA can simply disapprove MRT for any cases not involving mitochondrial disease correction. The FDA will also need to make recommendations on parental rights, and we agree with England’s vote to leave parental rights with the two nuclear donors. As a final safety precaution, the FDA could approve only protocols in which the male embryos are transferred (so if a problem occurs with MRT, the male’s mtDNA would not be passed onto their offspring), but we conclude there is no need for this.

Our overall recommendation is that the FDA approve MRT initially for a small number of patients, and follow their offspring’s progress closely for a few years before allowing the procedure to be done on a larger scale.

LITERATURE REVIEW

Part-1: Mitochondrial Diseases

Daniela Barbery

Introduction to Cells and Organelles

Humans, animals, and plants have a basic unit of life called the cell. Discovered by Robert Hooke, cells are the smallest unit capable of independent reproduction ([Nature.com, 2015](#)). Cells have different functions that are compartmentalized inside into different locations. In eukaryotes (including animals and plants), the functions are sorted into membrane-enclosed structures called organelles (**Figure-1**). Each organelle is important and works differently ([Ivyroses.com, 2015](#)). The nucleus controls the cell and stores the genetic information (DNA). The endoplasmic reticulum provides a highway for transporting molecules and contains enzymes for synthesizing lipids. Lysosomes contain hydrolytic enzymes that help digest biomolecules. The Golgi apparatus functions in secretion to help deliver molecules outside the cell. The organelle that is the focus of this IQP project is the mitochondrion (plural mitochondria). Its main function is the production of energy for the entire cell, provided as the molecule ATP. But mitochondria also have other important functions, such as calcium storage, regulating cellular metabolism, and apoptosis (programed cell death).

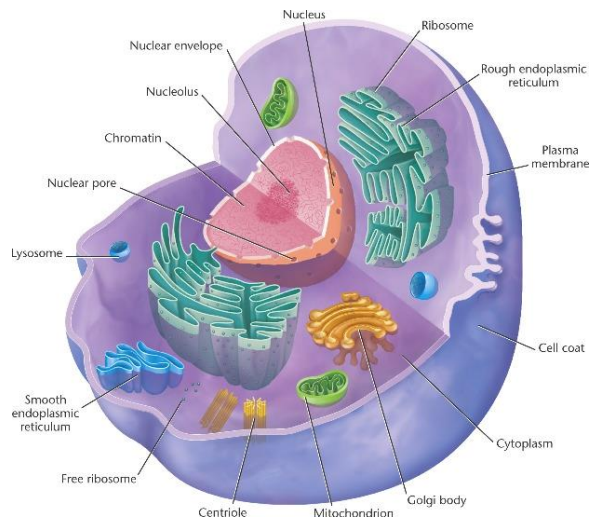


Figure-1: Diagram of a Typical Animal Cell. Shown are the major organelles within a typical animal cell, including the mitochondria (green in the diagram), the subject of this IQP project. Figure from: "Animal-cell.html 02_01-animal-cell.jpg." Animal-cell.html 02_01-animal-cell.jpg. Web. 17 May 2015.

Mitochondria Structure and Function

Mitochondria are the main organelles that provide energy to the cell. The number per cell vary widely, but in humans can vary from hundreds to thousands. Some cells can contain thousands of mitochondria. They are composed of an inner and outer membrane, each comprised of a phospholipid bilayer (**Figure-2**). The inner membrane is folded into cristae (upper white arrow in the diagram). Cytoplasm inside the inner membrane is termed the matrix. The cytoplasm between the two membranes is termed the intermembrane space. The inner membrane contains the enzymes responsible for using oxygen and chemicals provided from the cell's cytoplasm to perform cellular respiration to create ATP. The mitochondria are considered semi-self-sufficient organelles, since they have their own DNA and ribosomes to synthesize several of their own proteins.

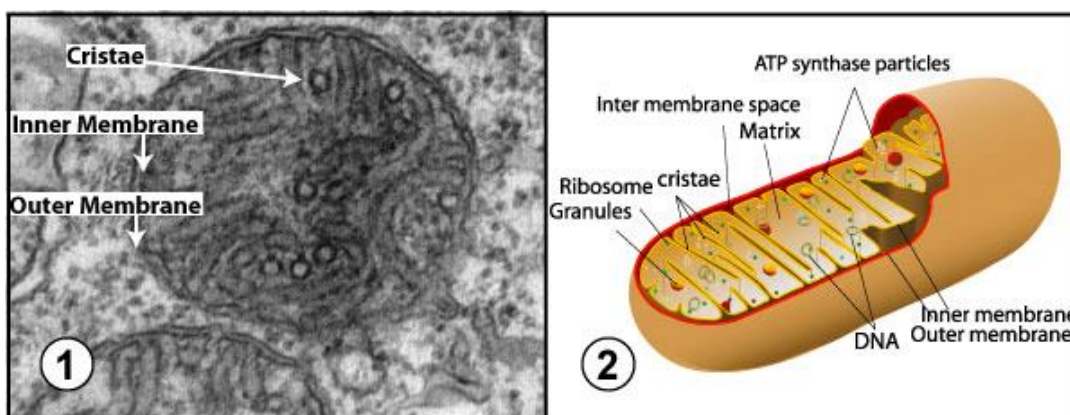


Figure-2: Photograph and Diagram of a Typical Mitochondrion. The left panel shows an electron micrograph of a typical mitochondrion, the main subject of this IQP project. The inner and outer membranes are denoted by white arrows. The right panel shows a diagram of the mitochondrial structure, including arrows to the cristae (inner membrane sites of ATP production) and mitochondrial DNA. Figure from: Bailey R (2015) "Mitochondria." Web. 17 May 2015. <<http://biology.about.com/od/cellanatomy/ss/mitochondria.htm>>.

The origin of mitochondria is still only a theory, but the most widely accepted model (the endosymbiont theory) suggests that present-day mitochondria resulted from an ancient symbiosis. This theory argues that the first mitochondrion was an aerobic prokaryote cell (capable of utilizing oxygen and pyruvate to produce energy) that was engulfed by a larger anaerobic eukaryote cell (by itself not able to utilize oxygen to efficiently produce energy). The advantage of the symbiosis was the smaller cell was provided nutrients and protection, while the larger cell was provided with an excellent source of energy. The endosymbiosis probably happened about two billion years ago. Over time, the engulfed prokaryote cell transformed into mitochondria (including the loss of some of its own genes into the cell's nucleus), therefore the ancient aerobe and present day mitochondria are significantly different. Because present-day *Rickettsia* (a proteobacteria) have DNA whose sequence closely matches present-day mitochondria, the theory argues that an organism similar to *Rickettsia* likely was engulfed in ancient times (Ferla et al., 2013).

Interaction Between Mitochondria and Nuclei

As discussed above, the most widely accepted theory on mitochondrial evolution states that present-day mitochondria evolved from ancient Rickettsia proteobacteria that were engulfed by a larger anaerobic cell. Because Rickettsia are free-living organisms, they have their own DNA. But over time, some of this DNA moved into the cell's nuclear DNA (Sykes, 2003), so that present-day mitochondria now contain only about 37 genes (about 16,600 base pairs), which is not enough to independently exist (Calloway, 2014).

The displacement of mitochondrial genes into the nucleus is an important topic for our IQP project on mitochondrial replacement therapy, because it dictates that a cell's mitochondria and nucleus must interact to properly function. The mitochondrial genes that moved into the nucleus encode proteins that must be synthesized and then delivered back into the mitochondria to function. In fact, the majority of mitochondrial proteins are synthesized outside the mitochondria and imported inside. So, the nucleus and mitochondria must constantly interact, and the proteins encoded by nuclear DNA must be compatible with (provide proteins that fully function) inside the cell's mitochondria. In September, a group of evolutionary biologists led by Klaus Reinhardt at the University of Tübingen (Germany), stated that “problems could arise if mitochondrial and nuclear DNA from different women proved to be incompatible”. They cited dozens of experiments in mice, fruit flies, and other animals where mixing nuclear and mitochondrial DNA from individuals with different genetic backgrounds sometimes led to problems such as reduced growth, early death, accelerated ageing or reduced reproductive ability (Hayden, 2013).

Mitochondrial DNA (mtDNA)

The human mitochondrial DNA (mtDNA) structure is a circular double-stranded DNA molecule, which contains approximately 16,600 base pairs (**Figure-3**). In humans, the number of mtDNA molecules varies, depending on the cell type, from 100 to 10,000 copies. mtDNA's strands are classified as the heavy strand (rich in the heavy base guanine), and the light strand (rich in the lighter base cytosine). The heavy strand encodes 28 genes, and the light strand encodes 9 genes, to make a total of 37 genes. The closed circular mtDNA structure is similar to that of all prokaryotic cells, including Rickettsia.

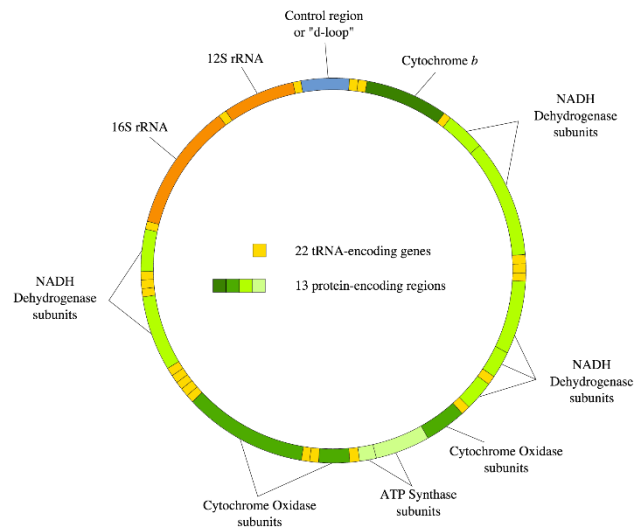


Figure-3: Diagram of Human Mitochondrial DNA. Shown are the main genes encoded by human mtDNA, including the genes encoding ribosomal RNAs (orange), proteins (green), and transfer RNAs (yellow). Figure from: "Basic Structures of Prokaryotic Cells - Boundless Open Textbook." Boundless. Web. 25 May 2015. <<https://www.boundless.com/biology/textbooks/boundless-biology-textbook/prokaryotes-bacteria-and-archaea-22/structure-of-prokaryotes-141/basic-structures-of-prokaryotic-cells-562-11775/>>

Mitochondrial genes can be classified into three main types: those encoding proteins (13 genes), those encoding transfer RNAs (22 genes), and those encoding ribosomal RNAs (2 genes) (Wikipedia, 2015).

The 13 protein encoding genes are:

MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6 (responsible for NADH dehydrogenase production).

MT-CYB (coenzyme Q: cytochrome c reductase (complex III)).

MT-CO1, MT-CO2, MT-CO3 (cytochrome oxidase (complex IV))

MT-ATP6, MT-ATP8 (ATP synthase (complex V)).

The 22 transfer RNA genes are: MT-TA, MT-TR, MT-TN, MT-TD, MT-TC, MT-TE, MT-TQ, MT-TG, MT-TH, MT-TI, MT-TK, MT-TM, MT-TF, MT-TP, MT-TT, MT-TW, MT-TY, MT-TL1, MT-TL2, MT-TS1, MT-TS2, MT-TV.

The two ribosomal RNAs genes are:

MT-RNR1 (12S)

MT-RNR2 (16S)

Mitochondrial Inheritance

Mitochondrial DNA is maternally derived (reviewed in: Fine, 1978). Humans reproduce sexually by a fertilization process, the union of a female egg cell pro-nucleus and a male sperm cell pro-nucleus. These cells contain haploid nuclear genetic information, 23 chromosomes each, to create a diploid zygote of a normal 46 chromosomes. The egg usually lets only one sperm penetrate its walls, and rapidly the egg disintegrates the sperm's head membrane and disposes of the tail. This means that the male flagellum, plasma membrane, and mitochondria located in the tail's midpiece (approximately 5 mtDNA molecules) are discarded and do not play a role in the new zygote's genetic material (Sato and Sato, 2013). Meanwhile the sperm head contains the haploid male pro-nucleus to combine it with the egg's haploid pro-nucleus. The egg contains around 250,000 mtDNA molecules. After fertilization, these mitochondria become the source of energy for the new embryo and the multiplying cells.

This maternal mitochondrial inheritance process means that mtDNA undergoes less genetic variance than nuclear DNA (which recombines with each new generation of offspring), and assists researchers to trace back in time a maternal family lineage. The procedure of analyzing mtDNA to analyze a descendant's lineage is usually performed by testing two hypervariable regions (HVR1 and HVR2). These genes (about 440 base pairs long) do not vary as much as nuclear DNA, but vary enough over many generations to map the changes to determine a mitochondrial haplotype and lineage.

Mitochondrial Diseases

Mitochondrial diseases are caused by mutations in mitochondrial DNA genes, or by mutations in nuclear genes encoding proteins that function within mitochondria, such as those involved in the electron transport chain (reviewed in Gropman, 2001; Thorburn and Dahl, 2001; Taylor and Turnbull, 2005; Reeve et al., 2008; Chinnery, 2015; Wallace, 2015). Because mitochondria are found in almost all cells in the body and they are extremely important in energy production, mitochondrial diseases can affect any tissue in the body. However, the diseases most commonly manifest in those tissues that rely strongly on energy production, such as brain, heart, muscle, pancreas, and kidney (Gropman, 2001). Mitochondrial diseases can affect a specific organ, or can affect a group of organs. There is no single age of onset.

Figure-4 shows two example inheritance pedigrees of a mitochondrial DNA mutation, indicating how a mitochondrial disease is passed to the offspring. In the upper pedigree, the female at the top with the mutated mitochondrial DNA (dark circle in the diagram), so all of her direct offspring (downwards lines) get the trait. The next generation offspring (third row) only inherit the disorder from second row females. The second pedigree shows a different scenario. As before, the first generation offspring (second row) all directly inherit the mutation, and the third row inherit only if the second row are females, but note that in the 3rd row only some of the offspring directly receive the mutation from the mother.

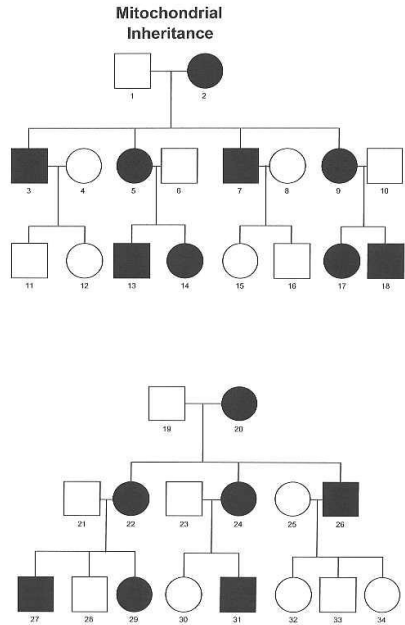


Figure-4: Two Example Pedigrees of Inheritance of a Mitochondrial DNA Disease. Circles denote females, and squares males. Filled in circles and squares denote diseased individuals. Figure from: Genetics Resource Center, "Mitochondrial Inheritance." MiGRC -. Web. 26 May 2015. <<https://migrc.org/Library/Mitochondrial.html>>.

With respect to treatments for mitochondrial diseases, they are typically limited to trying to manage symptoms than actually correcting the underlying genetic problem. For example, a physician might prescribe anticonvulsant drugs to treat seizures (Callaway, 2014), but the physician would not be able to correct the underlying mutation itself. Because of the current lack of cures for mitochondrial diseases, this IQP project focuses on a new technology termed mitochondrial replacement therapy, which may allow the mutation to be cured for the next generation of offspring (discussed in the next section).

With respect to prevalence, the incidence of mitochondrial diseases is difficult to gauge due to the diverse range of symptoms, and due to a “loose” genotype-phenotype relationship (some mutations cause a weak or no effect, while others cause very strong effects) (Schaefer et al., 2008). So it is no surprise that the published studies vary widely in their estimates. One study done by scientists at Newcastle University (UK) analyzing working age people in Northeast England found the incidence of mitochondrial disease at approximately 9.2 people per 100,000 (1 per 10,869), and found they represent the commonest form of inherited neuromuscular disorders (Schaefer et al., 2008). Another study done at the same university followed the frequency of 10 specific mtDNA point mutations in 3,168 neonatal cord blood samples comparing them to the mother’s blood sample, and found about 0.54% (1 per 185) of the offspring had mtDNA mutations (not all would cause disease), and that 0.00107% (1 per 93,457) contained new mutations not found in the mother (Elliott et al., 2008).

In another study, Gorman et al. (2015) calculated the number of women who could benefit from mitochondrial replacement therapy. They calculated the number of women with pathogenic

mtDNA mutations taking records on their fertility background, then compared that information to the general population's fertility background to check for differences. For women between 15-44 years of age in the U.S. and U.K., they found 63.2 carrier live births per 1000 person-years, while the general population was 67.2 (live births per 1000 person-years) so no significant difference in live birth rate for carriers (Gorman et al., 2015). They also estimated about 12,423 women in the U.S. in the same age range were at risk passing the mitochondrial disease to their offspring, predicting that these 12,423 women carriers would give birth between 4,574 to 23,354 times over a 30-year period. They also estimated the MRT procedure in the U.K. would help about 150 women each year.

Asymptomatic Carriers and Symptom Variations

The number of mitochondria per cell varies widely by organism, tissue, and cell type (Rogers, 2014). For example, human red blood cells have no mitochondria, while liver cells can have more than 2,000. The average human cell contains around 1,000 mitochondria. Because most human cells contain many mitochondria, and each mitochondrion has up to 10 copies of mitochondrial DNA (mtDNA), some human cells can acquire more mutated mtDNA than other cells. This randomness means that some women can be apparently healthy, while still carrying some diseased mitochondria that can be passed onto their offspring in the egg cell. The problem is sometimes not even discovered until giving birth to children who are very ill (Vogel, 2014).

Because brain, muscle, and heart require high amounts of energy, the symptoms of mitochondrial diseases often occur in those tissues first. And due to the type of mutation and the randomness of the ratio of mutated to beneficial mitochondria, some symptoms occur at birth, while other diseases become apparent only in adulthood.

Example Mitochondrial Diseases

1. Leber's Hereditary Optic Neuropathy

Leber's hereditary optic neuropathy (LHON) is one of the most common mitochondrial inherited disorders transmitted from mother to offspring. The disease was first described by the German ophthalmologist Theodor Leber in 1871 when he observed four families of young men who suffered from abrupt painless loss of vision (Leber, 1871). The sudden progression of LHON has a mean of 3.7 months. LHON causes a degeneration of the patient's retinal ganglion cells (RGCs) and their axons, leading to a loss of central vision. It predominantly affects young adult males (86% of cases), and only 10% are females. There is still no precise explanation for this gender bias, it could be due to anatomical, hormonal or physiological factors.

The disease was initially thought to be X-linked (associated with mutations on the X-chromosome), but was later shown to be mitochondrial (Erickson, 1972). It is now known to be mostly (>95% of cases) caused by mutations in the mitochondrial DNA at locations 11778 G to A (50-70% of the cases) (Wallace et al., 1988), 3460 G to A (8-25% of the cases) (Huoponen et al., 1991), and 14484 T to C (10-15% of the cases) (Johns et al., 1992), respectively, in the ND4, ND1 and ND6 subunit genes of complex-I of the mitochondrial oxidative phosphorylation chain (electron transport chain). LHON patients with the 14484/ND6 can experience spontaneous

recovery of about 36% in a period of 16 months, while patients with the 11778/ND1 mutation only have a 4% recovery rate.

LHON has multiple therapies, but they are usually inadequate. Idebenone is a medicine that helps restore electron flow in the mitochondria (disrupted by the mutation). This drug is a short peptide chain derived from coenzyme Q₁₀ and is used with vitamin B2 and C to stimulate ATP synthesis. This treatment shows random improvements in LHON patients. Other scientists are testing a type of gene therapy that uses adenovirus-associated vectors (AAV's) injected into the patient's eye (or animal model's eye) to deliver normal copies of the mutated mtDNA gene into the nucleus for expression in that location (allotopic gene therapy) (Koilkonda and Guy, 2011). The AAV vectors speed the process of transgene delivery and expression and reduce cellular degradations, but can cause an immune response in the patient which limits a second injection of virus. A big advancement for treating LHON was the development of a new procedure termed OCT (optical coherence tomography) that measures retinal nerve signals which decrease substantially with LHON (Koilkonda and Guy, 2011). This technique should allow earlier identification and treatment of LHON patients.

2. Pearson Syndrome

Pearson syndrome (PS) is a mitochondrial disease first described in 1979 by pediatric hematologist/oncologist Howard Pearson (Pearson et al., 1979). A decade later, it was found that the disease is caused by a deletion in mitochondrial DNA which removes the genes for cytochrome c-oxidase II and II and the transfer ribonucleic acid gene for serine and aspartic acid (Rotig et al., 1989). The disease is very rare, with less than hundred cases reported worldwide.

The symptoms include sideroblastic anemia (the bone marrow produces ringed red blood cells instead of healthy ones), pancreas and other organ dysfunctions, failure to thrive, insulin-dependent diabetes, muscle and neurologic impairment, and, frequently, early death. It is usually fatal in infancy. PS is characterized by ptosis, ophthalmoplegia, oropharyngeal and severe proximal limb weakness. It also causes neutropenia (low levels of white blood cells) leading to frequent infections. PS patients suffer of pancreas malfunction causing a lack of production of enzymes for digesting consumed fats, which eventually accumulate in the liver. Another vital function of the pancreas is to secrete insulin to maintain a correct blood sugar level, PS impedes the pancreas to properly function and patients often develop diabetes. Many other side effects lead to early death or low quality of life.

The exact location of the mtDNA deletions changes from patient to patient (DiMauro, 2015). Antibody analyses indicate that different respiratory enzyme complexes are affected. In a Pediatric Research Center in Nagoya City University Medical School, two Japanese patients with Pearson's Syndrome were tested for mutation sites (Sano et al., 1993). The first patient's deletion started at the ATP 8 gene to NADH dehydrogenase 5 gene. The second patient's deletion were in a different location on his mtDNA, from the transfer RNA to the cytochrome b gene. To diagnose the Pearson Syndrome is necessary to take a leukocyte DNA analysis test. In present-day there are no treatments to cure this syndrome, although sometimes patients sometimes benefit from an oral delivery replacement of pancreatic enzymes.

3. Kearns-Sayre Syndrome

Kearns–Sayre syndrome (KSS) was first described in 1958 in a case report of two patients by Thomas P. Kearns and George Sayre ([Kearns and Sayre, 1958](#)). The disease is also known as oculocraniosomatic disorder. Its symptoms typically appear before the age of 20. KSS is a multisystem mitochondrial syndrome that is symptomatically a more severe variant of chronic progressive external ophthalmoplegia (CPEO), a disease also discovered by Kearns and Sayre (1958). It usually affects the muscles controlling eyelid and eye movements, but can include cerebellar ataxia, proximal muscle weakness, paralysis, deafness, diabetes mellitus, growth hormone deficiency, hypo-parathyroidism, or other endocrinopathies ([Harvey and Barnett, 1992](#)). Other eye conditions also can have pigmentary retinopathy (degeneration of the retina), and loss of vision. Other symptoms include abnormal EKG signals to a complete heart block, high levels of protein in CSF that surrounds and protects the brain and spinal cord, and ataxia ([National Center for Biotechnology Information, 2015](#)).

KSS occurs spontaneously in the majority of cases, but in some cases it has been shown to be inherited through mitochondrial, autosomal dominant, or autosomal recessive mutations. There appears to be no preference for race or sex, and there are no known risk factors. Some identified mutations induce a respiration/ATP failure. Currently, there is no cure, but it is sometimes treated with implantation of a pacemaker to improve heart abnormalities, or by ingesting coenzyme Q10 to improve respiration.

4. Barth Syndrome

The Barth syndrome (BTHS) was named in 1983 in the Netherlands for pediatric neurologist Peter Barth for his description of a pedigree of an unusual inherited trait ([Barth et al., 1983](#)). The syndrome is sometimes termed 3-Methylglutaconic aciduria type-II syndrome. It is an X-linked genetic disorder found exclusively in males ([Claypool et al., 2008](#)), and appears to affect multiple organ systems. Females have a XX karyotype which helps them not manifest the disorder due to the second normal X-chromosome. Males on the other hand have a XY karyotype, which leaves no choice but to manifest the mutated-X symptoms. It is believed to be severely under-diagnosed ([Cantlay et al., 1999](#)), and is estimated to occur in about 1 of 300,000 births.

Barth syndrome is thought to be caused by mutations in the gene tafazzin (TAZ, also called G4.5) whose protein functions as an acyltransferase in complex lipid metabolism ([Vreken et al., 2000](#)). A 2008 study showed that all the Barth individuals tested had abnormalities in their cardiolipin molecules ([Kulik et al., 2008](#)), a type of lipid found inside mitochondria and intimately connected with the electron transport chain and membrane structure. The human tafazzin gene is located on the long arm of chromosome-X at site Xq28. Mutations in tafazzin that cause the Barth syndrome include missense, nonsense, deletion, frame shift, and splicing mutations. BTHS symptoms include dilated cardiomyopathy, endocardial fibroelastosis, growth retardation, neutropenia, proximal skeletal myopathy, and particularly the excess of 3-methylglutaconic acid. The syndrome creates a gap between the patients pre- and post-pubertal growth, which creates a remarkable abrupt difference. It is characterized by an enlarged and weakened heart muscles and

short stature. Males affected by BTHS have a reduced life expectancy, but those who survive the critical young years sometimes survive to their late forties.

BTHS diagnosis requires a series of evaluations, including a complete blood count, EKG reading, plasma amino acid analysis, and a medical genetics consultation (Ferreira, 2015). This syndrome does not have a cure, but there are symptom relievers. Medications to manage the acute heart decompensation include beta blockers, digoxin, ace-inhibitors, and IV inotropes. Aspirin is considered for prevention of blood clots, also arrhythmia medications or heart transplants have sometimes been successful. G-CSF can be administered to manage the neutropenia, but only in children. Nutrition is essential for preventing muscle loss.

5. Leigh's Syndrome

Leigh syndrome (LS) is a progressive neurodegenerative disorder first described by Denis Leigh in 1951 (Leigh, 1951), and was later distinguished from the similar Wernicke's encephalopathy in 1954. In 1968 Hommes et al. reported the disease's link with mitochondrial activity (Hommes et al, 1968), although the specific mutations in the cytochrome c oxidase and other electron transport chain proteins were not discovered until 1977.

LS symptoms include focal or bilateral lesions appearing in the central nervous system resulting from the abnormal production of energy. It results from mutations in the mitochondrial respiration chain complex: complex I (site 252,010), complex II (site 242,011), complex II deficiency (site 124,000), complex IV deficiency cytochrome c oxidase (site 220,110) or complex V deficiency (site 604,273) (Genetics Home Reference, 2015). LS also can result from mutations in nuclear genes encoding mitochondrial proteins. Typically the symptoms start at 3-12 months old, and are followed by retardation, hypertrophic cardiomyopathy, or peripheral neuropathy. About 50% of affected individuals die within 3 years from cardiac or respiratory arrest.

LS occurs in about 1 in 40,000 newborns. But in Quebec, Canada the incidence is more common at 1 to 2,000 newborns. LS can also derive from spontaneous mtDNA mutations without having a family history of the syndrome. Males and females both develop the pathology, but the inherited syndrome is an X-linked recessive pattern, so males are more prone to develop this syndrome than females. LS patients are sometimes treated with thiamine or Vitamin B1 supplements.

6. MERRF Syndrome

MERRF syndrome (Myoclonic Epilepsy with Ragged Red Fibers) is a very rare mitochondrial disease with an estimated prevalence of 1/400,000 in Northern Europe. The defining feature is the presence of "Ragged Red Fibers" in the muscle, that consist of clumps of diseased mitochondria that accumulate in the subsarcolemmal region of the muscle fiber and appear as red when the muscle is stained with modified Gömöri trichrome stain. Like most mitochondrial diseases, there is no cure for MERRF, and treatment is primarily symptomatic. Generally the first symptom is myoclonus (a brief involuntary twitching of a muscle), followed by other symptoms, such as: recurrent epilepsy episodes, ataxia, peripheral neuropathy, and dementia. The disease manifests during early years, including hair loss, short stature, and optic atrophy.

Over 80% of MERRF cases are caused by a maternally-inherited point mutation at position 8344 (Wahbi, K., 2010) in the mitochondrial genome which disrupts the mitochondrial gene for tRNA-Lys. This mutation has a broad downstream effect, disrupting the synthesis of proteins essential for oxidative phosphorylation. Other genes involved include: MT-TK (Zeviani et al., 1993), MT-TL1, MT-TH (Melone et al., 2004), MT-TS1 (Nakamura et al., 1995), MT-TS2, and MT-TF (Mancuso et al., 2004).

Diagnosis typically includes a leukocyte analysis, but multiple tests have to be run as MERRF can affect other tissues such as skin fibroblasts, urine sediment, oral mucosa, saliva, and hair follicles. There is no cure, but patients are sometimes treated with convectional medicines to mute the symptoms, including antiepileptic pills for seizures, physiotherapy and specific exercises for muscle impairments, and drugs to treat the cardiac symptoms (clonazepam or levetiracetam, etc.) (Wahbi, 2010). Other treatments are still in trials, including the consumption of Coenzyme Q10 and L-carnitine.

7. Friedreich's Ataxia

Friedreich's ataxia (FRDA) is an autosomal recessive inherited disease that causes progressive damage to the nervous system. The condition is named after the German physician Nikolaus Friedreich, who first described it in the 1860s. Its incidence in the general population is roughly 1 in 50,000. The disease is caused by a genetic mutation (expansion of an intronic GAA triplet repeat) in the FXN gene, which leads to a reduced expression of the mitochondrial protein frataxin. The ataxia portion Friedreich's ataxia results from the degeneration of nervous tissue in the spinal cord, especially sensory neurons essential for directing arm and leg muscle movements.

FRDA has a slow rate of progression, it begins around age ten, and almost always before age 25. Dysarthria, muscle weakness, tendon reflexes and paralysis in the lower limbs, vertebrae scoliosis and bladder malfunction are characteristics of FRDA. Two thirds of the patients suffer hypertrophic cardiomyopathy, a chronic heart muscle disease that enlarges and weakens it, and 30% develop mellitus diabetes. As the disease progresses, FRDA patients usually depend on a wheelchair. This disease, as mentioned above, is inherited in an autosomal recessive manner; therefore it is not dependent on gender. The disease has a pedigree pattern in which any offspring from the same generation as the affected individual has a 25% chance of also being affected, a 50% chance of being an asymptomatic (no symptoms) carrier, and a 25% chance of not having the mutation.

Treating this type of disease consists of ingesting multiple drugs and medical interventions, so these patients often require psychological support and counseling. For each symptom there is a corresponding treatment: limbs prosthetics for walking aid or in extreme cases a wheelchair. Tendon paralyses are treated with pharmacologic drugs. For scoliosis orthopedic intervention is required. The cardiomyopathy therapy uses cardiac failure medications, anticoagulating agents, and if needed a pacemaker implantation. For individuals who also suffer from diabetes, a dietary modification is obligatory and insulin injections. For bladder dysfunction, an antispasmodic is prescribed (Bidichandani, 2014).

Part-1 Conclusions

Currently, there are no cures for mitochondrial diseases, so doctors can only attempt to alleviate some of the symptoms. Women carrying mitochondrial mutations have three current options:

1. They can adopt a baby, not genetically related to father or mother.
2. They can undergo a modification of traditional IVF that uses a donor egg (including its nucleus) and the father's sperm, to make an embryo not related to the mother.
3. They can use prenatal testing on their own IVF embryo to try to determine whether the embryo inherited any diseased mitochondria.

New experimental therapies might include:

1. Performing gene therapy with a virus encoding the non-mutated gene, delivered by an adeno-associated virus (AAV) to the patient's nuclear DNA in the tissue most strongly affected. Although this would need to be done prior to any serious damage to the tissue.
2. Try MRT protocol discussed in this IQP, which would benefit the offspring, but not the original patient.

Overall, this chapter shows that some mitochondrial disease syndromes are caused by mutations in mitochondrial DNA itself, and offspring from these females could in theory benefit from MRT. But other patients with the same syndromes can result from mutations in the nuclear DNA encoding mitochondrial proteins, and these mothers' likely would not benefit from MRT. So, prior to performing MRT on any patient, tests should be performed to determine where the mutation lies. This chapter also shows that some of the mutations in mtDNA can occur spontaneously, with no family history of the disease, so these females would need to be diagnosed and treated with MRT to benefit their offspring.

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Part-2: Background on IVF and Infertility

Emily Caron

As many as one couple in six eventually encounter fertility problems, defined as the failure to achieve a clinical pregnancy after regular intercourse for 12 months. So increasingly, couples are turning to assisted reproductive technology (ART), especially in vitro fertilization (IVF) technology, for help conceiving. This IQP project focuses on a new method of IVF termed mitochondrial replacement therapy (MRT) for potentially curing mitochondrial diseases. Because MRT technology has many of the same biological and technical problems likely to be encountered by MRT patients, this section provides information on IVF technology and its problems.

Natural fertilization involves regular intercourse followed by penetration of the egg by the sperm. Fertilization usually occurs in the fallopian tubes as the egg moves towards the uterus, and this is considered as day-0. Following fertilization, as discussed in the Literature Review part-1, the mitochondria of the sperm midbody are destroyed (leaving only the female mitochondria in the egg cytoplasm, female mitochondrial inheritance). The haploid male pronucleus fuses with the female haploid pronucleus to make a diploid embryo. Embryo implantation into the uterine wall usually occurs at around day-5 (Bavister, 2002).

In contrast, *in vitro* fertilization (IVF) is a type of ART in which an egg is fertilized by sperm outside the body. The process involves several key steps: 1) monitoring and stimulating a woman's ovulatory process, 2) surgically removing an ovum or ova (egg or eggs) from her ovaries, 3) mixing the egg with sperm (or injecting sperm into the egg) to allow fertilization, 4) culturing the fertilized zygote for 2-6 days in a growth medium to create a cleavage-stage embryo or a blastocyst, and 5) implanting the embryo into the woman's uterus (or that of a surrogate) to hopefully establish a pregnancy. Several variations of IVF exist, including the use of a pregnancy surrogate, the use of donated eggs, or the use of donated sperm. IVF is frequently used to overcome female infertility resulting from blockage of the fallopian tubes, where the eggs no longer successfully move to the oviducts to become fertilized. IVF can also be used to assist with male infertility, especially in cases of low sperm quality or quantity. In some cases, the sperm is directly injected into the egg cytoplasm using intra cytoplasmic sperm injection (ICSI), which increase the success rates. IVF is also sometimes performed to avoid the passage of genetic diseases to offspring, such as when performing preimplantation genetic diagnosis (PGD) to screen embryos prior to implantation. If approved in the U.S., a new type of MRT would be done, mitochondrial replacement therapy (MRT), the subject of this IQP.

In vitro fertilization is now a well-established technology with a variety of applications in basic and applied sciences. IVF treatments can now routinely provide higher quantities of good quality eggs, and higher percent fertilization success. However, as we will discuss below, IVF technology has several problems, and the criteria used to judge the results of IVF experiments can be misleading. For example, success rates are often reported as the percent of treated individuals giving live births, but few experiments follow the health of the offspring long-term (Bavister, 2002).

IVF History

IVF was adapted to humans only after years of animal research. People often forget the importance of animal research in today's scientific advancements. IVF origins occurred in basic research studies conducted as long ago as 1878. Between 1878 and 1953 numerous attempts were made to fertilize mammalian eggs in vitro. Many reports were published during that time claiming they had achieved success, but these previous claims appear to be unjustified. They typically used eggs that were too immature to become fertilized ([Edwards, 1996](#)).

The 1950's and 1960's were arguably the most productive years for IVF development. A major advance in 1951 concluded that the most probable cause for the earlier failures (where sperm was injected into the uterus) was the spermatozoa were introduced to the egg *during*, rather than *prior* to, ovulation. When sperm was introduced *prior* to ovulation, fertilization rates improved dramatically. Thus, scientists theorized the spermatozoa must reside in the female reproductive tract for some time before acquiring the capacity to penetrate eggs. From this, the term *capacitation* was formed. The discovery of the importance of sperm capacitation renewed interest in IVF. Discoveries were made about the important biochemical reactions that occur in sperm after ejaculation which prepare them for egg penetration. After the discovery of sperm capacitation, labs began reporting fertilization successes (not necessarily live births) for several animal species including rabbits, mice, hamster, rats, and humans ([Bavister, 2002](#)).

In 1961, Italian scientist Daniele Petrucci claimed to have 40 eggs that he successfully fertilized and grew in the lab for 29 days until the embryo had a heartbeat and then he destroyed it. However, many scientists were skeptical of Petrucci's claims, because at that time, no one could grow a human embryo that long in vitro. At the same time, two British scientists, Robert Edwards and Patrick Steptoe, fertilized a human egg in vitro, grew it for four days, and then implanted it into a uterus. But this experiment failed after ten weeks due to fetal development in the fallopian tube rather than the womb ([Congressional Research Services, 1976](#)). However, after nearly one hundred failed attempts with human embryo transfers, the British team finally developed a successful protocol for human IVF ([for a review see Edwards, 2001](#)), and in 1978 produced the first successful human "test tube baby", Louise Joy Brown, born in England on July 25, 1978 ([BBC News, 1978](#)). Her mother used natural cycle IVF (no hormonal stimulations) to induce ovulation. Louise is currently 37 years old (in 2015), and has had a son of her own ([BBC News, 2007](#); [Moreton, 2007](#)). Both Louise and her son are closely being watched medically for any IVF side effects. In 2010, Robert Edwards was awarded the Nobel Prize in Physiology or Medicine for his development of IVF ([NobelPrize.org, 2010](#)).

The second successful birth of a test tube baby occurred in India, just 67 days after Louise Brown was born. The girl, named Durga, was conceived via IVF using the methods of Subhash Mukhopadhyay (a physician and researcher from Kolkata). Thereafter, IVF technology rapidly expanded. Improvements increased the number and quality of eggs harvested, the use of ultrasound to examine the follicles to determine optimum harvesting time, the development of oocyte aspiration procedures, and techniques to monitor blood flow to the ovary and uterus ([Edwards, 1996](#)).

IVF Methods

Ovulation

Although IVF in theory could be performed using a woman's natural ovulation cycle and taking the egg directly from a woman's fallopian tubes or uterus, this procedure rarely works, so the woman is instead injected with hormones to create ovarian hyper-stimulation. Ovarian hyper-stimulation induces the development of multiple eggs at a single coordinated time. This method can also include the suppression of spontaneous ovulation, which helps coordinate the ovulations to the desired time (La Marca et al., 2014). This generates multiple eggs (10-30) that are retrieved either surgically through the abdominal wall, or by ultrasound-guided trans-vaginal oocyte retrieval directly from the ovaries (La Marca and Sunkara, 2014). Injectable gonadotropins, usually follicle stimulating hormone (FSH) analogs are used to start the process. The progress is closely monitored to check serum estradiol levels, and ultrasound to monitor follicular growth. Typically 10 days of initial injections are necessary. Using hyper-stimulation, for women aged between 40–42, England's Human Fertilization and Embryo Authority (HFEA) (that oversees IVF procedures) has estimated the live birth rate increases from approximately 1.3% per IVF cycle for natural ovulation to 3.9% when using hyper-stimulation (HFEA, 2015).

Mild IVF is a method where a small dose of ovarian stimulating drugs is used for a short duration, producing from 2–7 eggs (Nargund, 2009). The purpose of mild IVF is to reduce complications while improving egg quality. One study showed that live births were about 43.4% using mild stimulation compared to 44.7% with standard stimulation (Heijnen et al., 2007). Mild IVF can be cheaper than conventional IVF with a reduced risk of Ovarian Hyper-Stimulation Syndrome (OHSS).

After the initial series of injections, the follicles have reached late development. Their final maturation is induced by an injection of human chorionic gonadotropin (hCG), commonly termed the "trigger shot." If left alone, ovulation (rupture) would occur 38-40 hours after a single HCG injection, however for IVF purposes the eggs are retrieved prior to rupture about 34-36 hours post-injection. Thus, the eggs can be retrieved from a known location (the follicle) at a time they are fully mature. The hCG trigger shot comes with a risk of ovarian hyper-stimulation syndrome, so some scientists use GnRH instead of HCG, even though it decreases the egg retrieval by 6% compared to hCG (Humaidan et al., 2011).

Egg Retrieval

Egg retrieval can be performed 34-36 hours after the final injection but before ovulation (Killick, 2006). The eggs are usually retrieved from the patient under conscious sedation or general anesthesia using a trans-vaginal technique that takes 20-40 minutes. Transvaginal oocyte retrieval is more properly referred to as transvaginal ovum retrieval once the oocytes have matured into ova, as is normally the case in IVF. Ultrasound is used to guide a needle through the vaginal wall to reach the ovaries, taking care not to injure organs located between the vaginal wall and the ovary. The other end of the needle is attached to a suction device. Once the follicle is entered, suction is gently applied to aspirate follicular fluid and ideally the egg is extracted with it. Once the ovarian follicles have been aspirated on one ovary, the procedure is repeated on

the other ovary. Usually 10-30 eggs are removed by aspiration through the needle. It is not unusual to remove this many oocytes due to the fact that women are commonly hyper stimulated before the procedure.

If the ovaries are not accessible by transvaginal oocyte retrieval, then abdominal surgery is performed using laparoscopy. In this procedure, a tiny incision is made near the navel, and a slender viewing instrument (laparoscope) is inserted to help guide the needle for retrieval.

Mature eggs are placed in a culture medium and incubated. Eggs that appear healthy and mature will be used for IVF, however not all eggs retrieved will become fertilized (Killick, 2006).

Co-Incubation of Egg and Sperm

In the lab, the donated eggs are prepared for IVF by removing the surrounding cells, and selecting those eggs the physician believes are most hardy. The semen is washed by removing inactive cells and seminal fluid. When sperm is provided by a donor, it is already washed and frozen, so must be thawed. The sperm and egg cells are incubated together at a ratio of about 75,000:1 in a culture medium. Incubating only 1-4 hours appears to result in higher pregnancy rates than incubating 16-24 hours (Zhang et al., 2013). In situations of low sperm count or motility, a sperm cell is directly injected into the egg by intra-cytoplasmic sperm injection (ICSI). If the egg has become fertilized, it will show two pronuclei (male and female). The fertilized egg is passed to a special growth medium and left for approximately 48 hours until the egg consists of six to eight cells.

The *in vivo* fertilization equivalent of co-incubation would be gamete intra-fallopian transfer. With this, eggs are removed from the woman and placed in one of her fallopian tubes along with the man's sperm. This allows fertilization to take place inside the woman's body (Zhang et al., 2013).

Length of Embryo Culture and Embryo Selection

The fertilized egg is placed in a special growth medium, and cultured to the cleavage stage (2-4 days) or the blastocyst stage (5-6 days). Culturing to the blastocyst stage increases the live birth rate, but because some embryos die during culture, it also decreases the number of embryos available for transfer. Using blastocyst embryos instead of cleavage embryos also appears to significantly increase the odds of preterm birth (less than 37 weeks) and may increase congenital anomalies (Sroga et al., 2010; Dar et al., 2014).

Various grading methods exist for selecting the embryos most likely to survive, however a morphological scoring system appears to be the most reliable strategy (Rebmann et al., 2010). Since 2009, when the first time-lapse microscopy system for IVF was approved for clinical use, morpho-kinetic scoring systems have been shown to improve pregnancy rates.

During embryo culture, couples who may have a high risk of passing a genetic (hereditary) disorder to their child sometimes consider pre-implantation genetic diagnosis (PGD). This procedure is done approximately 3-4 days after IVF prior to implantation. A single cell is removed from each cleavage stage embryo, and that cell is screened for the specific target

genetic disorders using polymerase chain reaction (PCR). PGD can help determine which embryos are healthiest, significantly decreasing the chance of passing a disorder to the child. These healthy embryos are then implanted. However, PGD is considered controversial and is not offered in all clinics ([Rebmann et al., 2010](#)).

Embryo Transfer to the Uterus

Embryo transfer refers to the IVF step where the embryos are placed into the uterus of a female with the intent to establish a pregnancy. Embryos are chosen for transfer based on the amount of cells, evenness of growth, and degree of fragmentation. Embryos are usually transferred to the woman's uterus using a thin, plastic catheter inserted through the vagina and cervix. The number of embryos transferred depends on the total number available, the woman's age, and the woman's overall health. Some countries limit the number of embryos that can be transferred. Canada, the UK, Australia and New Zealand, allow a maximum of two embryos (except in unusual circumstances). The UK may allow women over 40 to receive up to three. In contrast, the United States allows younger women to have many embryos transferred based on individual fertility diagnosis. Due to pregnancy problems, many physicians wish to minimize the risk of carrying multiples during pregnancy, while other physicians wish to transfer multiple embryos to increase improve the chances of implantation and pregnancy ([Mains and VanVoorhis, 2010](#)). IVF is expensive (see below) so more than one egg is usually transferred to increase the chance of success, but the number must be regulated to minimize the risks associated with multiple pregnancies (see below). Unused embryos may be frozen and implanted later, donated to other couples, or donated for research purposes.

Adjunctive Medication

Following embryo transfer, hormones are sometimes administered to aid embryo growth and implantation into the uterine wall ([Van der Linden et al., 2011](#)). Luteal support is the administration of medication (typically progesterone, progestins, or GnRH agonists) to increase the success rate of implantation and early embryogenesis. These drugs complement and support the function of the corpus luteum. The use of progesterone or co-treatment with GnRH agonists leads to an increased birth rate. However, other growth hormones or aspirin have no evidence of overall benefit.

IVF Success Rates

Due to advances in reproductive technology, IVF success rates have improved significantly in recent years. Success rates are usually classified as confirmed pregnancies (% pregnancy rate) or live births (% live birth rate). The live birth rate does not include miscarriages or stillbirths, and twins/triplets are counted as one pregnancy. Because each initiated IVF cycle does not necessarily end with oocyte retrieval or embryo transfer, reports of live birth rates need a qualifier (i.e. success per number of IVF cycles started, per IVF retrievals, or per embryo transfers). Multiple IVF attempts improve the odds. One study reported live birth rates as high as 51%-80% when the mother underwent six attempts ([Neighmond, 2009](#)).

The main factors affecting IVF success appear to be maternal age, length of the infertility, and the number of oocytes collected ([van Loendersloot et al., 2010](#)). The success

rates strongly decline with age. For example, the U.S. Society for Reproductive Medicine (2012) reported the average IVF success rates as follows, where the decline with age is very evident:

Age:	<35	35-37	38-40	41-42	>42
Pregnancy Rate	46.7	37.8	29.7	19.8	8.6
Live Birth Rate	40.7	31.3	22.2	11.8	3.9

In another study, in 2008, several Canadian clinics reported an overall live birth rate of about 27%, and as expected the success rate declined with age. Patients 21 and younger showed a success rate of 35.3%, patients near 37 year old showed 27.4%, and 0.0% live births were observed for women older than 48 in this particular study ([de La Rochebrochard et al., 2008](#)). Some clinics exceed these rates, but it has not been determined whether this increase is caused by superior technique or careful patient selection. Success rates can be improved by refusing patients whose cases would be more difficult.

The success rates also improve when fresh eggs are used versus frozen eggs. For example, the statistics from the Society for Assisted Reproductive Technologies ([SART, 2015](#)) provided the following live birth rate comparison of fresh and frozen-thawed eggs:

Donor Eggs:	Fresh	Thawed
Live Birth Rate:	55.1%	33.8%

Using IVF, women past their reproductive years can still become pregnant. Adriana Iliescu initially held the record as the oldest woman to give birth using IVF and a donated egg, when she gave birth in 2004 at the age of 66 (although that record was later exceeded in 2006). Although it is possible to become pregnant by IVF at this late age, because the success rates are so low, most fertility clinics in the U.S. limit the eligibility to 50-55 years. Overall, while IVF technology can sometimes overcome infertility in older women, it does not reverse the underlying age-dependent decline in fertility ([Appel, 2009](#)).

While physical factors (like age) highly influence IVF success, many clinics also keep an eye on the mental toll that these treatments have. Stress plays a large role in the success rate of IVF treatments. In a 2005 Swedish study, 166 women were monitored for psychological factors starting one month before their IVF cycles and through the IVF process. The results showed a significant correlation between psychological stress and IVF outcome ([Canadian Press, 2008](#)). The study recommended that it might be possible to reduce patient stress during IVF by informing them of their findings. The financial burden of IVF treatment alone can also lead to high levels of anxiety.

IVF Usage

With respect to how often IVF technology is used, Israel has the highest rate of IVF in the world, performing roughly 1,657 procedures per million people annually ([Gallagher, 2013](#)). Iceland holds a close second with 899 procedures performed per million people per year. The reason for Israel's high usage rate may result from the government's support of unlimited free IVF procedures for its citizens for up to two children for women under the age of 45. But in

many other countries the coverage of IVF procedures is limited or non-existent (Gallagher, 2013).

In the U.S., approximately 126 IVF procedures are performed per million people per year, but its utilization highly varies and increases with the availability of trained physicians and IVF insurance coverage, and somewhat increases with the percentage of single persons and with median income (Hammoud et al., 2009). In 2009, in the U.S. on average there were approximately 2.5 IVF physicians per 100,000 (Hammoud et al., 2009).

IVF Costs

IVF is an expensive and time consuming process. There are both pre-treatment expenses and IVF treatment costs. Pretreatment expenses include initial appointments, costs for specialists, and tests such as blood tests, semen analysis, and ultrasounds. The IVF treatment costs include blood tests, pathology, ultrasounds, standard medications, counselling during and immediately post cycle, fertility specialist consultations, specialist care, and support.

In the U.S., the average cost of IVF in 2011 (from egg retrieval to embryo implantation) was approximately \$12,400 (Kraft, 2011). Most insurance companies do not pay for IVF treatments, and those that do pay often cap the number of cycles (Kraft, 2011). The costs are even higher per live births, being \$41,000 in the U.S., \$40,000 in the U.K., and about \$24,500 in Scandinavia and Japan (Chambers et al., 2009).

In other countries, IVF costs are typically lower. For example, in Japan it costs approximately \$4,000, and in Ireland about €4,000 (Chambers et al., 2009). Israel, with their very high IVF rates, spend only about \$3,450 per procedure. Comparing the U.S. and other countries, the cost per delivery in 2001 in the U.S. averaged \$56,419 compared to \$20,522 in eight other countries. Multiple gestation births significantly increase the cost IVF treatment. IVF multiple births cost about 36% more than the IVF treatment itself. Most studies indicate that infertile couples would increase their usage of IVF services if the price were lower, and the high cost especially keeps the procedure out of reach in many developing countries (Gallagher, 2013).

Summary of IVF Problems and Questions

The types of problems encountered by IVF patients will likely also be encountered by MRT patients (the subject of this IQP project), so a summary of the problems is shown below. Some of these problems will be followed up in interviews to use experts in the IVF field to help prioritize which topics are likely to be most problematic.

1. Side Effects: IVF patients undergoing supplemental hormone treatments, such as GnRH, sometimes develop side-effects such as ovarian hyper-stimulation syndrome. This can be minimized by using a different hormone (hCG), but this decreases the live birth success rate by 6%. Will MRT patients also encounter potential side-effect problems with ovarian hyper-stimulation syndrome? Is this syndrome easily treatable?
2. Patient Selection: Some IVF clinics show increased success rates by limiting which patients are enrolled for their IVF procedures. MRT patients are different than typical IVF patients, and can suffer serious symptoms from their mitochondrial disease. Will severely diseased

women showing strong symptoms from their mitochondrial disease be healthy enough for MRT?

3. Stress: Stress has shown to decrease IVF success rates. How can patient stress be minimized during MRT?
4. Age: IVF success rates strongly decline with increasing age of the patient. If this also pertains to MRT women, will MRT be performed mostly on younger women?
5. Prenatal Genetic Diagnosis: PGD has been used during IVF to screen for genetic mutations using PCR tests prior to embryo transfer. PGD can help determine which embryos are healthiest, and those embryos are the ones implanted. Will PGD be used with MRT to ensure the embryo to be implanted contains no diseased mitochondria?
6. Cost: IVF is a very expensive procedure. In the U.S., IVF is not covered by most medical insurance plans. Will MRT (involving 3 people, not 2) cost even more than IVF? Will medical insurers likely treat MRT (and its potential to eliminate a fatal disease) differently than IVF (where the patient is looking to have a baby), to where MRT patients can gain access to the procedure?
7. Equipment: Some studies suggest that doctor training and use of specific types of equipment can improve IVF success rates (the success rates vary depending on the clinic). Is this also expected to be the case for MRT?

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Part-3: The MRT Procedure

Benjamin Grondin

The technique touted as a potential cure for mitochondrial disease in the offspring is called **mitochondrial replacement therapy (MRT)**. Some press reports refer to the procedure as producing 3-parent embryos, but this term is actually a misnomer because only 2 of the 3 individuals retain parental rights (the donor of the healthy mitochondria retains no parental rights). During MRT, the nuclear DNA from the mother (with mitochondrial disease) is extracted from an egg (hopefully leaving behind all her mutated mitochondria) and this nuclear material is transferred to the enucleated egg of a female with healthy mitochondria in its cytoplasm. That egg is then fertilized with the father's sperm. As with all IVF procedures, the egg is then cultured either to the cleavage stage or to the blastocyst stage, and is then implanted into the mother's uterus.

To some individuals, this procedure is highly controversial because it creates embryos with "new DNA" (referring to the addition of the healthy mitochondrial DNA). But other individuals argue the MRT technique is similar to "changing the batteries on a car" (referring to the role of mitochondria in energy production). This section of the Lit Review focuses on the way the MRT procedure is performed, and describes some of the experiments done with it to date.

Human eggs are about 0.1 mm in diameter, so nuclear transfers must be done under a microscope with a specially designed chamber to control temperature and humidity (Callaway, 2014). Sometimes the mother's egg (providing the nucleus) is zapped with a laser to temporarily make a hole in its membrane to allow a pipette to be inserted to extract the nucleus. The same laser procedure is used to enucleate the donor egg (providing the healthy mitochondria), and the micropipette is used to inject the mother's nucleus. The injection procedure usually takes several minutes.

First Suggestion for Using MRT

The first published suggestion for using human MRT (by nuclear transplantation) to treat mitochondrial diseases was made in 1995 by a team of scientists at Loyola University (Chicago), published as a Comment in the *Cambridge Quarterly of Healthcare Ethics* (Rubenstein et al., 1995). The authors suggested that *in vitro* ovum nuclear transplantation could be used to transfer the nucleus from a diseased egg (containing diseased mitochondria) into an enucleated egg containing normal mitochondria, and they discussed the ethics of performing the transfer technique as it would alter the "genetic structure" of the egg.

A second suggestion to use nuclear transplantation to treat mitochondrial disease came in 1999 from Richard M. Roberts, then of the Genetics and Prenatal Diagnostic Center (Signal Mountain, TN). In a Letter to the Editor of the *American Journal of Medical Genetics* (Roberts, 1999), he stated that the idea actually originated from Bruce Wallace 10-15 years prior to the 1999 article, but Wallace's idea was never published. Roberts raised the concept of treating mitochondrial diseases in view of the successes with nuclear transfer technology in animals,

discussing some of the ethical considerations of this type of “human cloning”.

Early Animal Experiments

As is usually the case in biology, human experiments are rarely conducted without first performing animal experiments. In the case of MRT technology, the early experiments were performed mostly on mice (with some experiments on sheep), and predominately focused on potential problems with heteroplasmy (having two different types of mitochondria in one embryo). For example, in 1997 a study was conducted in mice ([Meirelles and Smith, 1997](#)) to test how long heteroplasmy lasts in mammals and what mechanisms are present that segregate mitochondria into daughter cells during cell division. Mouse lines were created containing two different genotypes of mitochondria, and then they were bred to each other. The offspring were observed over many generations. The first generation exhibited heteroplasmy as expected, but within two generations some offspring were found to be homoplasmic (one type of mitochondria). In other offspring, the two kinds of mitochondria were found to have fused to make intra-organelle heteroplasmy, which was permanently transmitted to their offspring. Of note, in one of the heteroplasmic females, the level of heteroplasmy varied from tissue to tissue, indicating that the segregation mechanism likely takes place early during development, likely during meiotic division. So, this paper shows that mitochondrial heteroplasmy once created can indeed be passed to offspring, and will be an important topic to follow up on in interviews. Could a better understanding of the mitochondrial segregation help us develop better techniques to counteract any heteroplasmy that might occur during MRT procedures?

In 2004, Bai and Wong induced heteroplasmy in mice and observed the behavioral changes relative to homoplasmic mice. The heteroplasmic mice showed a reduced physical activity, decreased appetite, and a more dramatic stress response than the homoplasmic control group. Cognitive impairment was also evident in the heteroplasmic mice as tested in a Barnes Maze Test, characterized by a slower learning time. So, this experiment indicates that mitochondrial heteroplasmy can affect offspring, and is worth following up in interviews in this IQP project. The conclusion that heteroplasmy can cause alterations in behavior and cognition was also observed in a more recent study ([Sharpley et al., 2012](#)).

In 2005, scientists at the University of Tsukuba (Ibaraki, Japan) created transgenic mice that mimic a mitochondrial disease with respiratory effects ([Sato et al., 2005](#)). They performed a large-scale deletion of mitochondrial DNA (Delta-mtDNA) in their “Mito-mice” strain to mimic the mitochondrial disease, then showed that the respiratory defect could be reversed in IVF offspring by performing nuclear transplantation into eggs with normal mitochondria. This experiment provided a proof-of-principle that nuclear transplantation can be used to treat a mitochondrial disease.

In 2007, an experiment was performed by a Canadian research team to determine the physiological effects of heteroplasmy in mice ([Acton et al., 2007](#)). Tests were set up to assay cardiovascular and metabolic functions, hematological parameters, body mass, ovarian reserves, and tissue histological abnormalities between the heteroplasmic mice and a control set of homoplasmic mice. The mice were raised for fifteen months with tests taken at regular intervals. Heteroplasmic mice were found to have significantly higher heart rates at a young age, had increased body and fat mass indexes, and contained abnormal electrolytes and hematological

factors. These tests give validity to the concern that some scientists raise about heteroplasmy during MRT procedures. When it comes to doing human MRT, do scientists intend to assay for the extent of heteroplasmy in the offspring? Genotypes can be monitored in the IVF embryo by PCR prior to implantation, do they intend to do this to select embryos that have no mitochondrial carryover from the mother?

Mitochondrial heteroplasmy was also found to occur naturally during nuclear transfer cloning procedures (which directly relates to our MRT topic of this IQP). For example, a study done in sheep ([Burgstaller et al., 2007](#)) found that cloned sheep (like cloned mice) show evidence of heteroplasmy caused by mitochondrial carryover during the procedure. The carryover was usually relatively low (0.1% to 0.9%, n = 6), but one sheep showed 6.8% to 46.5% heteroplasmy.

Early Human Ooplasmic (Egg Cytoplasm) Transplantation Experiments

Prior to using nuclear transfer to treat mitochondrial disease, a team of scientists led by Jacques Cohen at a fertility clinic in New Jersey (The Institute for Reproductive Medicine and Science of Saint Barnabas, Livingston, New Jersey) performed ooplasmic (egg cytoplasm) transplantation experiments to attempt to treat infertility in 7 couples with multiple implantation failures ([Cohen et al., 1997 and 1998](#)). Although the women could make enough eggs for IVF (they were not old) “their eggs were a mess, the cytoplasm around the nucleus was fragmented and littered with debris” ([Callaway, 2014](#)). Dr. Cohen wondered what would happen if he added a little cytoplasm from another woman’s healthy egg. They injected less than one picoliter (10^{-12} liter) of another woman’s egg cytoplasm into 33 previously infertile eggs. Of the 7 original couples, two embryos showed improved morphology after egg cytoplasm injection, both embryos resulted in pregnancies, but one miscarried. A third pregnancy resulted from an embryo with no obvious morphology improvements. Two other patients did not become pregnant, according to the authors likely due to poor donor embryos. Another embryo produced one baby. And the last embryo produced a pregnancy that was ongoing at the time of the 1998 publication. Overall, 17 babies were born from this study and a later one. Unwittingly, they became the world’s first genetically modified humans. Some of these children have shown serious health problems, so continue to be monitored.

In 1999, scientists at The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School (Norfolk, VA) did an experiment to determine whether the cytoplasm from frozen eggs could be injected into recipient eggs taken from women with a history of infertility or over the age of 40 to improve their fertility ([Lanzendorf et al., 1999](#)). Eggs were collected from 4 donors and cryopreserved. 61% (28/46) survived the freezing and thawing procedure. Fertilization occurred in 70.3% (26/37) of the injected embryos, and one twin pregnancy resulted from a 35-year old patient with a history of poor embryo quality. This study indicates that eggs can likely survive freezing and thawing, eliminating the need to coordinate donor and receiving eggs. These babies unknowingly were also genetically altered by the mitochondrial injections, as with the New Jersey babies.

In 2000 and 2001, scientists at the same location in New Jersey as the earlier 1997 and 1998 papers discussed above (Institute for Reproductive Medicine and Science of Saint Barnabas, West Orange, NJ) used mtDNA fingerprinting to assay for mitochondrial

heteroplasmy (cells containing mitochondria from two different donors). They found positive heteroplasmy in the following tissues (parentheses indicates positives relative to total samples tested): embryos (6/13), amniocytes (1/4), placenta (2/4), and fetal cord blood (2/4) (Brenner et al., 2000). They also analyzed the blood of two of their babies born from embryos injected with egg cytoplasm, and both babies tested positive for heteroplasmy (Barritt et al., 2001). This evidence proves that both types of mitochondria (original and donor) survived in the baby's cells, and provides the first hard evidence of human "germline" genetic modification, resulting in "apparently normal" children. The children's health is only now being monitored for long-term effects.

The health status of these early ooplasm-injection babies is uncertain. The children are now teenagers, but have not gone through middle-age. Some of the animal experiments discussed above show that MRT mice develop hypertension and obesity in middle age, and show impaired cognition. One of the ooplasm-injection babies developed autism, and two fetuses developed Turner Syndrome (one miscarried and one aborted) (Callaway, 2014). Dr. Cohen (now at Reprogenetics in Livingston, NJ) is teaming up with his former colleagues at Saint Barnabas for a follow-up study on the children, including phone surveys of their health, and saliva tests to assay for heteroplasmy (Callaway, 2014). These follow-up studies should provide useful data for the ongoing debate about MRT. The team stopped performing ooplasm-injection experiments in 2001 when the US FDA determined that more animal research is required before mitochondrial injection can be used in humans.

In 2001, Dr. David Whitehouse in an article for *BBC News* (Whitehouse, 2001) discussed the early ooplasm-injected children experiments. He indicated that while the babies born from these programs appeared to be healthy, the techniques permanently changed their human germ line. Any changes to the human germ line, no matter how small, are deemed to be highly controversial, and the program would have been illegal in other countries at the time. The mitochondrial injection technique was also looked upon by Lord Winston of the Hammersmith Hospital as an experiment "without evidence of whether it was worth doing", and seen by Professor Joe Cummins (University of Western Ontario, Canada) as a way of bringing "human germline therapies" though the back door (Whitehouse, 2001).

Human MRT

In 2009, Dr. Shoukhrat Mitalipov and his team of scientists at the Oregon National Primate Research Center (Beaverton, OR) in their paper entitled "Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells" established a preclinical monkey model for testing MRT protocols (Tachibana et al., 2009). Using their "spindle replacement" protocol, they transferred the spindle-chromosomal complex (containing the nuclear genes) from a female monkey (in the future equivalent to a person with a mitochondrial disorder) to an enucleated donor egg (with normal mitochondria). The reconstructed egg was shown to support normal fertilization with male sperm, exhibit normal embryo development, and produced 3 apparently healthy offspring (two were named "Mito" and "Tracker" after a lab reagent used to make mitochondria glow) (Callaway, 2014). The monkeys were 5 years old in 2014 and were still apparently healthy according to interviews with Dr. Mitalipov (Callaway, 2014). Genetic analyses showed the 3 offspring monkeys had nuclear DNA from one female, and mitochondrial DNA from another female. Importantly, their assays found no mitochondrial DNA from the

nuclear donor (no heteroplasmy), so in theory this spindle replacement technique could be used in the future on humans, and might not generate any mitochondrial heteroplasmy (cells containing two types of mitochondria) which some scientists argue can produce problems (Sharpley et al., 2012).

In 2010, Dr. Mary Herbert's group at the Newcastle Fertility Centre at Life (UK), in their paper entitled "Pronuclear Transfer in Human Embryos to Prevent Transmission of Mitochondrial DNA Disease" (Craven et al., 2010), used human IVF embryos to show that transfer of the pronuclei between fertilized zygotes results in small amounts (about <2%) carryover of mitochondrial DNA, and allowed apparently normal embryo development *in vitro* at least to the blastocyst stage. Many of the embryos contained no detectable donor mtDNA, so the authors claim the MRT technique has the potential to treat mitochondrial diseases. But this study showed that some of the embryos did indeed contain 2% heteroplasmy, which other scientists worry might cause a problem, so perhaps this means that the MRT embryos should be screened for heteroplasmy prior to implantation? Perhaps we need more scientific studies in mice with different percentages of heteroplasmy to determine the maximum amounts that produce no negative effects? And questions still remain as to whether this human procedure would be efficient enough to result in a live pregnancy when performed on relatively low numbers of human embryos.

In 2013, Dr. Mitalipov's team at the Oregon National Primate Research Center followed up their earlier paper with another study entitled "Towards Germline Gene Therapy of Inherited Mitochondrial Diseases" (Tachibana et al., 2013). In this paper, the authors used their previously termed "spindle replacement protocol", now termed a "spindle transfer" (ST), to study donated human oocytes. Of 106 human oocytes donated for research purposes, 65 underwent ST, and 33 were normal IVF controls. The IVF fertilization rate was similar in both groups, but the ST group showed elevated numbers of abnormal fertilizations as evidenced by irregular numbers of pronuclei. Development to the blastocyst stage and the isolation rates of embryonic stem cells (ESCs) from the blastocysts were similar between the two groups. All derived ESCs from the ST group showed normal karyotypes and appeared to contain exclusively donor mitochondria (again no evidence of mitochondrial heteroplasmy). Thus, it appears that mitochondria can be efficiently totally replaced in human eggs, and are capable of normal development to the blastocyst stage. In an interview with the authors, it would be interesting to determine which assays were used to monitor for potential heteroplasmy, and whether they are capable of detecting heteroplasmy at rates as low as 1%.

In another study performed in 2013, Dr. Dieter Egli's team at the New York Stem Cell Foundation Laboratory (New York, NY), entitled "Nuclear Genome Transfer in Human Oocytes Eliminates Mitochondrial DNA Variants" (Paull et al., 2013), demonstrated the feasibility of performing nuclear genome transfer to prevent the transmission of mitochondrial disorders in humans. The scientists removed the nucleus from the oocyte of a donor female (in the future one with mitochondrial disease, hopefully leaving behind all her mitochondria) and fused that nucleus with an enucleated oocyte (containing normal mitochondria in the cytoplasm). The MRT procedure did not appear to reduce the efficiency of development to the blastocyst stage. Importantly, contaminating mtDNA was positively detected in this study. Initially the contaminating mtDNA was detected at about 1%, but after further embryo cell divisions and development it decreased to undetectable levels in blastocysts and embryonic stem cell (ESC) lines derived from the blastocysts. There was no measurable contaminating mitochondria

detected after a year of cell passaging, including manipulations to induce differentiation into a variety of different types of cells. Importantly the mitochondria from the procedure had respiratory chain enzyme activities and oxygen consumption rates comparable to mitochondria from the original donor cells, so the MRT mitochondria appeared to be functional. This is an interesting study showing that although mitochondrial heteroplasmy might exist following MRT in cleavage stage embryos, the heteroplasmy soon disappears following cell divisions and cell differentiations to the blastocyst stage. It was not clear from the data presented whether the eggs studied were fertilized with sperm, or just artificially fertilized.

In a 2014 article titled “The Challenges of Mitochondrial Replacement” (Chinnery et al., 2014) several leading scientists from Newcastle University and the Oregon National Primate Research Center addressed the concerns raised by several scientists that there might be an evolutionary relationship between mtDNA and nuclear DNA which could cause unexpected issues when different genotypes are matched during MRT. They cited several diseases that could potentially be cured by MRT, including LHON (Liber’s hereditary optic neuropathy), but were not convinced of any meaningful matching problems. No mitochondria-nuclear matching problem would cause a disease as serious as LHON, but they noted that several other studies saw other types of problems. They cited a study done on MRT macaques (serving as a close model to humans) where they had intentionally taken monkeys that were not highly genetically related, and all the MRT offspring showed no differences from the controls for health problems. But they did not monitor for long-term health problems. The article also brings up the fact that each male birth automatically tests for mitochondrial compatibility problems, because the male baby’s new nuclear DNA (recombined from the mother and father’s DNA) now exists in a new tandem with the mother’s mitochondrial DNA. They stated “*From the mitochondrial DNA perspective, any mitochondrial transfer experiment is just recapitulating what is happening every day all around the world—and without any known adverse effects*”. The article’s responses made sense, but it was unclear how their claims match with the known health problems of other studies, and most of their claims were speculative, so more research needs to be done to prove their points.

Correction of Mitochondria Disease Patients with Stem Cells

The MRT technique was developed to prevent mothers with defective mitochondria from passing the disease to their *offspring*, but would not help the patient. Very recently (July 15, 2015) a new technique was developed to potentially treat *current* mitochondrial patients with stem cells (Ma et al., 2015). Dr. Shoukhrat Mitalipov’s team (discussed previously, at the Oregon Health and Science University) created stem cells from Leigh’s syndrome and MELAS patients using two different techniques: 1) For patients containing no healthy mitochondria, they performed nuclear transfer on the patient’s egg to inject her nucleus into a healthy donor egg, but instead of fertilizing it and implanting the manipulated embryo into the uterus (as with MRT), instead they chemically stimulated the egg to start dividing, grew the embryo to the blastocyst stage, and then isolated pluripotent stem cells from the blastocyst. Those stem cells are genetically identical to the patient, and in theory could be used to replace some of her tissues with healthy tissue derived from the stem cells. 2) For patients containing a mix of healthy and diseased mitochondria, the team derived a different type of stem cell termed an “induced pluripotent stem cell” (iPSC) from the patient’s skin fibroblasts. The patient’s fibroblasts were treated with genes to reprogram them to de-differentiate into iPSCs. The iPSCs are also genetically identical to the patient and, in theory, could be used to treat diseased tissues. As the

iPS cells were cultured, the mitochondria were randomly distributed into the daughter cells, so some iPS cell lines contained mostly healthy mitochondria, while other cell lines contained mostly diseased mitochondria. The team then selected for those iPS cell lines that had the healthiest mitochondrial metabolism. This second iPSC approach, does not use “cloning” or “nuclear transfer” in any way, and does not alter the patient’s germ line, so perhaps it would be more acceptable to society and more readily approved than MRT.

Unfortunately, the stem cell technique is not yet ready for the clinic to treat patients. Dr. Robin Lovell-Badge of the Francis Crick Institute in London stated, “It’s going to be difficult to introduce [the stem cells] in a way that you would help a [mitochondria] patient. You’ve got to substantially replace the cells of the patient” (Yandell, 2015). And Dr. Michael Teitell at UCLA noted, “I think that anyone who is generating reparative stem cells has the same problem...how to get them into the body if you are going to use them as cell therapy” (Yandell, 2015).

Although the stem cell technique might not be ready for the clinic, the study did provide some interesting data on the mitochondrial heteroplasmy problem (Ma et al., 2015). The second approach (generating iPS cells from patients having both healthy and diseased mitochondria) created a variety of stem cell lines, each with different degrees of heteroplasmy. So, these cell lines could allow detailed controlled studies to be performed on cells with the same nuclear material and different degrees of heteroplasmy. And the first approach (using nuclear transfer) also provided a variety of cells to study, and none of them showed any problems with heteroplasmy. There was no evidence of small amounts of diseased mitochondria carry-over into the healthy egg. As stated by Dr. Robin Lovell-Badge (mentioned above), “They deliberately looked for evidence of incompatibilities...and they found none. That’s reassuring for the notion of doing nuclear transfer” (Yandell, 2015).

Problems and Questions

1. Heteroplasmy: Early experiments with mice show that mice engineered to contain two different genotypes of mitochondria (heteroplasmy) have reduced physical activity, decreased appetite, and a more dramatic stress, cognitive impairment, higher heart rates at a young age, were heavier on body and fat mass indexes, and had abnormal electrolytes and hematological factors. Will heteroplasmy be a problem with human MRT?
 - a. Percent Heteroplasmy: The extent of heteroplasmy likely will be important. Will 1% heteroplasmy be a problem in human MRT procedures if 1% of the mother’s mitochondria carry over into the donor egg? Do we need more studies with mice engineered to contain various amounts of heteroplasmy to know the acceptable percent that generates no downstream effects?
 - b. Changes in Heteroplasmy During Development: Some studies performed on human IVF embryos indicate that although mitochondrial heteroplasmy exists following MRT in cleavage stage embryos, the heteroplasmy soon disappears after cell divisions and cell differentiations at the blastocyst stage. So, will this result apply to all MRT embryos, and heteroplasmy if present will decline during development?

- c. Screening: If heteroplasmy is shown to cause problems, should we pre-screen embryos using PGT (prenatal genetic testing) to determine which embryos lack heteroplasmy, and implant only those embryos?
 - d. Genotype likely will be important. Are some genotypes of mitochondria likely to be more problematic matching nuclear genotypes than others?
 - e. Procedures: Some scientists see no evidence of heteroplasmy (for example when performing spindle replacement procedures), so are some techniques less likely to generate heteroplasmy than others?
2. Long-Term Health Problems: Since MRT is a relatively new technique, no study has monitored long-term potential health problems into middle age. The earliest mitochondrial injection experiments done in New Jersey in 1997 produced 17 offspring that are now 18 years old. Of those 17 babies, one developed autism, two fetuses developed Turner Syndrome, one miscarried, and one aborted (Callaway, 2014). Should scientists monitor the long-term health of MRT patients?

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Part-4: Ethics of IVF and MRT

Daniel Eckler

Assisted reproductive technology is a hot topic both for the medical scientific community and for any anguished couples who dream of become the parents of a healthy child. Unfortunately, the traditional means of conception fail for some couples, leaving them searching for answers, which sometimes causes them to seek IVF treatments. As discussed in previous sections, mitochondrial disorders are caused by mutations in mitochondrial DNA, and some of these disorders are fatal. Mitochondrial Replacement Therapy (MRT) is a new technique proposed to treat mitochondrial diseases by substituting the nucleus of the mother's egg (containing mutated mitochondria) into a healthy enucleated donated egg, and fertilizing the egg with sperm from the father. MRT potentially provides couples with mitochondrial disease a way to prevent passing the disease to their offspring. But MRT is a type of IVF, and comes with the same set of medical, ethical, and legal problems. Different religious communities question IVF and MRT techniques and their morality. The scientific community still debates the legitimacy of evidence showing whether the procedure is safe. This section of the Literature Review will focus on the ethical issues of IVF and MRT.

Religious Views of IVF

The Catholic, Muslim, and Jewish communities have various stances on the morality of IVF techniques. In 2008, the Congregation for the Doctrine of the Faith instructed members of the Catholic community on how to respond to issues of in vitro fertilization, embryo transfer, embryo donation, and genetic engineering through the release of the *Dignitas Personae*. The writing declared that, "the dignity of a person must be recognized in every human being from conception to natural death" ([Congregation for the Doctrine of Faith, 2008](#)). The Catholic community recognizes a human as living immediately after conception, so an IVF embryo in that view has rights as a living entity. But embryos developed for IVF are not always used by the parents, and may eventually be disposed, or used for research purposes with the consent of the parents. According to both the *Dignitas Personae*, and a speech by Pope Benedict XVI in 2006, an embryo has the right to be developed, so obviously the disposal of embryos in the laboratory contradicts this viewpoint. The *Dignitas Personae* reiterates that methods of "re-establishing the normal functioning of human procreation" cannot be used when the destruction of human beings themselves, or their rights is a result of the laboratory practice. Furthermore, with regards to infertility treatment, all techniques must respect three principles outlined in the article: 1) an embryo has the right to life immediately after conception, 2) marriage binds two human beings to only procreate with their spouse, and 3) the creation of a human being must result from the conjugal act of a married couple's love ([Congregation for the Doctrine of Faith, 2008](#)). So, IVF conflicts with most Catholic doctrine. Disposal of unused embryos challenges the first point if the embryo is not implanted. Techniques involving the use of a third party donated egg (to provide healthy mitochondria) challenges the second point. In 2006, Pope Benedict XVI appearing before the Pontifical Academy for Life stated his opposition to IVF as "it replaces the love between a husband and wife" ([Pope Benedict, 2006](#)). And the third point is largely up for debate, determining whether a couple chooses to utilize scientific methods out of love is difficult to gauge by an outside observer. Nonetheless, the Catholic community accepts infertile couples, and proposes adoption given those circumstances ([Catechism, 1993](#)). The Catholic Church

permits the use of natural family planning, such as charting ovulation times, and it allows the use of specific reproductive technologies that facilitate conception from normal sexual intercourse, such as using a fertility lubricant.

The Muslim community has long debated the topics of infertility, IVF, and other infertility treatments. Until recently, women struggled with approval from their culture and religion to approach clinics with their infertility issues. In his article published in 2014, Azadeh Moaveni describes the scene of an Iranian fertility clinic where women filled the clinic waiting room and shared stories of their troubles and travels to seek help there. Moaveni pointed out the fatwa (an Islamic scholarly article or instruction) framed on the office wall reassuring the women pursuing assisted reproductive technologies that they have the approval of their religion. But in reality, the Muslim community is far from unanimous on its opinion on IVF. For example, the article cites doctors in Saudi Arabia as being even more conservative than Iran, and explaining that they would never allow clinics to do IVF.

In the Muslim community, the pressure on women to produce children results in self-loathing individuals when they are unable to do so. Interestingly, Iran's Islamic family law states that, "babies born of sperm or egg donation fall into the legal category of adopted children and stepchildren, who are not permitted to inherit property from non-biological parents" (Moaveni, 2014). Even within a country portrayed by the author as the fertility treatment capital of the Muslim Middle East, morality and instruction on IVF is unclear. Nonetheless, a short article published by the IVF Worldwide website outlines Muslim views in a few short points. The first being that the husband's sperm must be used, and implying that the mother's egg must be used. Next, the preservation of embryos is allowed, as long as they are utilized during the marriage. Selective reduction, the removal of one embryo when multiple embryos are implanted, is allowed only if the mother's health and life are in danger. Lastly, the article states that only a licensed physician may perform IVF (IVF Worldwide, 2012). Thus, the Muslim community remains largely torn on the IVF issue.

A reoccurring theme with religion and artificial fertilization techniques articulates itself beautifully in an article published by Durham University (Dain, 2009). Doctor Sherman Silber, director of the Infertility Center of St. Luke's Hospital in St. Louis, explains how contradictions happen all the time with religions as modern science develops. Religious articles outlining the views of its members often fail to keep current with the fast paced IVF industry, much less provide clear and understandable instructions. For instance, Dr. Silber explains that "orthodox Jewish beliefs view the egg donor [if different than the wife] as interfering in the couple's marriage" (Dain, 2009). However, ovary transplantation is allowed. The difference leaves the average mother confused and uncertain. In both of these instances, the DNA of the child results from the egg donor, but in the latter technique the egg ovulates inside the mother's body, so ovarian transplant is acceptable. An article written by Dr. Silber featured on the US National Library of Medicine website goes into detail about how the Jewish community perceives infertility. Principally, the article states that as members of the Jewish faith, "we all have an obligation to have offspring and to be fruitful and multiply" (Silber, 2011), so fundamentally, Judaism allows nearly all forms of fertilization. Interestingly, the article explains that Jewish doctrine deems that the soul does not enter the embryo until 40 days after conception. Therefore, many questions about IVF morality or ethics are debunked by this one principal, because the IVF embryo would not have a soul until long after implantation into the uterus. For example, in Judaism selective reduction is an acceptable method of fertilization as long as the goal remains

enhancing the possibility of life, and the reduction takes place before 40 days. Although Orthodox Judaism remains an anti-abortion religion, 40 days presents the time needed for a human to exist, so even abortions could be performed within this time period. Technicalities sprout throughout topics of infertility, but the Jewish obligation to reproduce renders much of artificial fertilization acceptable.

IVF Birth Defects

The topic of IVF-induced birth defects is one of the most controversial topics in the IVF field. Although some studies have shown an increased risk of specific disorders associated with IVF, other studies argue the risks are slight and may instead be associated with the underlying infertility problems not the IVF technique. The scientific community focuses on the risks and potential hazards of infertility treatments. Though they have a different scope on the matter than religious scholars, the divisions remain equally intense.

A study conducted on behalf of the European Society of Human Reproduction and Embryology shows evidence of increased birth defects when using assisted reproductive technologies (Hansen et al., 2013). According to the article, evidence suggests, "a 32% increased risk of birth defects in children born following ART compared with non-ART infants, and this risk increases slightly when singleton births are examined separately (36%) and when the results are restricted to studies examining major birth defects only (42%)" (Hansen et al., 2013). The investigation gathered data from 3,963,431 infants, of which 92,671 were born using assisted reproductive technologies. Furthermore, the study considered the cause of the increased rate of birth defects, and the link to underlying infertility. For example, factors such as, "the medications used, culture media composition, length of time in culture, freezing and thawing of embryos, altered hormonal environment at the time of implantation, the manipulation of gametes and embryos, or a combination of these" may contribute to the increased risk. Interestingly, the article mentions that there is growing evidence of low-birth weight or preterm birth due to, "the transfer of frozen-thawed embryos or the use of different culture media" (Hansen et al., 2013). Simply put, the methods and procedures used by the scientists in the laboratory may lead to an increase in birth defects. Perhaps, reevaluating the assisted reproductive technology practices could close the gap that this study suggests between naturally born offspring and their counterparts.

Furthermore, a study published in 2012 found an increased risk of obstetric and perinatal complications in singleton pregnancies involving the use of IVF (Pandley et al., 2012). Most notably, when compared to a 1.00 risk for spontaneous pregnancies, the study suggests that the relative risk of Ante-Partum Syndrome by IVF was 2.49. The relative risk of perinatal mortality was 1.87, congenital anomalies was 1.67, low birth weight was 1.65, and Caesarean section was 1.56. The report also advises that pregnancies administered using assisted reproductive technologies should be managed as high risk (Pandey et al., 2012).

In addition, a team lead by Dr. Mert Ozan Bahtiyar found evidence to show that twins conceived through IVF techniques show higher incidence of congenital heart defects (Bahtiyar et al., 2008). Identical twins conceived through IVF, "showed up to a 13-fold increase in congenital heart defects" (Bahtiyar et al., 2008). The reasons for these defects are largely up for debate, but

when multiple embryos are implanted in the mother, the chance of multiple pregnancies and problems increase.

Not all studies show an increase in birth defects with IVF. Some scientists find the correlation between IVF and birth defects to be inconclusive, attributing the correlation more to underlying patient factors (such as the problems causing the infertility) instead of the procedures themselves. Barbara Dolinska addresses the opponents of assisted reproductive technologies in her article published for the Polish Psychological Bulletin in 2009. She argues that a systematic examination of all children born using assisted reproductive technologies is difficult because of cultural stigmas and restrictions ([Polish Psychological Bulletin, 2010](#)). For example, the countries of Belgium and Holland both refund parents for their procedures, and social approval is high, so in those countries, “medical examination of children is largely accepted by the parents”. In other countries where the costs of ART procedures are not refunded (Poland), and “where the procedures are not readily available and the very question of infertility is treated in terms of intimacy violation (Poland, Greece), people’s inclination to submit to such examination is lower, and the credibility of the obtained data is more doubtful” ([Polish Psychological Bulletin, 2010](#)). Thus, cultural barriers prevent researchers from gathering data from the entire population. Missing portions of the sample cause inaccuracies and diminishes the credibility of the data. With that in mind, Dolinska goes further explaining that, “The main reason for the weaker health of these children is often connected with the advanced age of the parents who choose IVF and their health condition” ([Polish Psychological Bulletin, 2010](#)). For instance, as much as “96% of Swedish IVF parents agreed to their own health evaluation, but in Greece only 25% of the IVF parents agreed to the evaluation.” Once again, failure to obtain data from the entire target population causes inaccuracies and diminishes the credibility of the data. Dolinska makes note of important factors when examining assisted reproduction such as, “the parents’ characteristics (including: the cause of their infertility), and prior infertility treatment (especially stimulation of multiple ovulation).” She attributes the parents own health conditions to the poor outcomes of children conceived through assisted reproductive technologies. According to the article, women utilizing IVF are 3 to 6 years older on average than women conceiving naturally ([Polish Psychological Bulletin, 2010](#)). Furthermore, the article marks women over 35 years old as a much higher risk for birth defects. Of the reported mothers that conceived naturally from 1997-2003, only 12.9% were older than 35 years. Of the reported mothers who utilized assisted reproductive technologies from 1997-2003, 44.9% were older than the age threshold. When comparing the pregnancies of the women older than 40 years old to those 20-29, the correspondence between age and pregnancy complications is quite apparent. The frequency of mal-presentation pregnancies was 11% for the former group and just 6% for the latter. The frequency of gestational diabetes was 7% for the former, and 1.7% for the latter. Minimum fetal growth restriction occurred in 2.5% of the pregnancies of the older group, and only 1.4% of the younger group. ([Polish Psychological Bulletin, 2010](#)) The data leads Dolinska to conclude that older women in general are taking advantage of assisted reproductive technology, and that older women simply experience pregnancy complications at a higher rate than younger mothers, not that the IVF procedures themselves are causing the problems.

Roger Hart and Robert J. Norman speak much of the same inaccurate and inconclusive data used to prove a link between assisted reproductive technologies and birth defects ([Hart and Norman, 2013](#)). They argue that data suggesting that autism increases with IVF is invalid because the, “incidence of autism, or at least the tendency to make a diagnosis, appears to have increased over the last three decades making any association with IVF treatment difficult to

derive” (Hart and Norman, 2013). They also concluded that IVF does not appear to affect child cognitive development, school performance, social functioning, or behavior. They pointed out the difficulty diagnosing some of the conditions assayed for, including autism, depression, and ADHD.

It is important to keep in mind that a majority of IVF babies live to become grown adults, so assessing their development could reveal the true value of assisted reproductive technologies to society. A study conducted by Jane Squires and Paul Kaplan focuses on the developmental problems of assisted reproductive technology offspring. The development of a child is usually assessed by administering standardized tests made for children. The study finds the growth of “children in terms of height and weight to be falling behind that of naturally conceived children during the first 3 years of life—probably because of their smaller size at birth” (Squires and Kaplan, 2007). Though physical attributes may lag behind with these children, the article goes on to explain that the motor and cognitive development of these children does not differ much from that of a naturally conceived child. Furthermore, the study suggests IQ scores tend to be influenced more by the mother’s “level of education and socioeconomic status than to the mode of conception” (Squires and Kaplan, 2007). In a global study, 300 singleton children born as a result of assisted reproductive technologies were tested against “naturally conceived children at 5 years, and no significant differences were found in cognitive development or psychological well-being”. The study concludes that physical problems are the main concern with current procedures, and suggests that concerns may subside with new technologies and better laboratory practices (Squire and Kaplan, 2007).

Preimplantation Genetic Diagnosis

One method scientists utilize to maximize the child’s potential of being fully healthy when the parents are carriers of gene mutations is preimplantation genetic diagnosis (PGD). This process analyzes IVF embryos for potential genetic disorders prior to transferring the embryo to the mother’s uterus. One cell is removed from an IVF embryo at the cleavage stage (about 8-cells) the DNA is extracted, and it is subjected to tests such as polymerase chain reaction (PCR) to screen for genetic diseases. If the embryo is found to have the genetic defect, it is often not implanted. The ethics of this procedure are interesting, and offer to some scientists a way to minimize genetic diseases.

Penn Medicine suggests that PGD may be recommended when the parents show a history of heritable genetic disorders in their families, or the mother has a history of miscarriages (Penn Medicine, 2015). Embryos discovered to carry chromosomal abnormalities are documented, and the parents are informed of the findings. The moral fate of these embryos leads right back to religious and scientific ethics, and whether the embryo is determined to be “unfit for life” and possibly discarded. The article reassures that the goal of PGD pertains to ensuring the embryo has the highest potential to develop into a fully functioning, healthy human being. However, parents may use the procedure to weed out genetic conditions that some doctors consider are not serious, or even to ensure that desirable traits are passed onto their child. In fact, a deaf British couple intended to use embryo screening to ensure their deaf trait would be passed onto their child (Lawson, 2008). Though powerful when used for the right reasons, PGD could eventually lead to eugenics where it is not just used to eliminate a particular genetic disease, but to eliminate

undesirable traits and sculpt offspring through a process of elimination in a laboratory. So far, PGD has been used to screen for genes related to Alzheimer's disease and Cystic Fibrosis. So, although using PGD to test for non-disease-related genes has not yet presented itself, Hamish Anderson of the University of Otago argues that, "If, hypothetically, sex selection for social reasons is allowed in New Zealand, could women claim to be devalued if the main use of this practice was to prevent the implantation of embryos with a female genotype? Within this changing environment expressivist objection may gain renewed traction" (Anderson, 2012). Similarly, would the elimination of embryos containing genes for blond hair, ambidexterity, or attached earlobes be morally unjust for society? Embryo screening lays the foundation for such practices, and as IVF PGD technology develops, the debate will surely continue.

MRT Ethics

MRT is a specific type of IVF, so it comes with a similar set of ethical issues as discussed above. Depending on the type of mutation, mothers carrying mitochondrial mutations tend to carry weak mitochondria, and fertilization can be difficult for them, so many will revert to IVF. As will be discussed in the next section of the Literature Review (MRT Legalities), Britain is currently leading the way in terms of investigating MRT, assaying the key issues surrounding the technology, and passing legislation allowing it soon to be performed on patients with mitochondrial diseases. In 2011, Britain's Human Fertilization and Embryology Authority (HFEA) conducted a scientific and ethical study on MRT (HFEA Review, 2011). The review concluded that MRT is potentially safe and ethical, but they focused on three key issues:

1. Is the procedure scientifically safe? The HFEA review concluded that MRT appears to be scientifically safe, but acknowledged that some IVF problems are associated with performing the procedure on middle-aged women.
2. Who retains parenthood rights? During the U.K. 2015 debate in the House of Commons, several members noted that the term 3-parent embryos is wrong, because the egg donor (providing the healthy mitochondria) is not granted any parental rights, and the donor identity will not be disclosed to the children (Parliament, 2015).
3. Does MRT constitute a germline modification that crosses an ethical "red line" that will result in its being used to treat problems other than fatal mitochondrial diseases? This problem is discussed below.

Within the U.S., the Food and Drug Administration (FDA) has formed an Ad Hoc Committee of the Institute of Medicine (IOC) to study the ethical and scientific issues involved in MRT. Their goal is to issue a consensus report by April of 2016 (U.S. FDA, 2014; The National Academies, 2014; Vogel, 2015). The U.S. has informally discussed some MRT ethical issues in the popular press, but until the IOC Report is issued, the U.S. has not published a formal study of the MRT technique (Cohen et al., 2015).

MRT and Modification of the Germline

The main ethical reason MRT is controversial is it modifies the DNA of the embryo such that it is passed on to future generations. Previous types of human gene therapy experiments corrected a specific gene for one individual's DNA for a specific genetic disease. In those cases,

the risks were assumed only by a consenting individual, usually a patient who had already exhausted all other forms of treatment for their fatal or debilitating disease. But with MRT, according to Marcy Darnovsky, Executive Director of the Center for Genetics and Society, “Unlike experimental gene therapies where risks are taken by consenting individuals, [MRT] turns children into our biological experiments, and forever alters the human germline in unknowable ways. There is no precedent for this” (Vogel, 2015). Until now, procedures that produced inheritable gene alterations have been ethically taboo (Vogel, 2014). UK conservative Parliament member Jacob Rees-Mogg equates MRT with cloning, and said “the technique would promote eugenics; in a country nervous about genetically modified crops, we are making the foolhardy move to genetically alter babies” (Callaway, 2014). In the U.S., in March of 2013, a group of ethicists sent a letter to *The Times*, stating that MRT would “open the door to further genetic alterations of human beings with unforeseeable consequences” (Callaway, 2014).

But other scientists point out that MRT would only be used to treat serious mitochondrial diseases, not play with the human genome. They argue that mtDNA makes up only a tiny fraction (0.1%) of the patient’s total genome, with little influence over a person’s *defining* traits (Callaway, 2014). Sometimes, human studies are the only way to obtain the correct information on safety, and we just have to proceed forward. Animal models are imperfect, and *in vitro* cell studies cannot provide information on long-term side effects, so we just need to move forward. Shoukhrat Mitalipov and Don Wolf published an article in 2014 addressing MRT issues, and suggested that mitochondrial DNA’s contribution to the individual’s total genome “is small, constituting just 0.1% of the total DNA. Moreover, the sequence variation between different mitochondrial haplotypes in the human population is small translating to just a few amino acid substitutions” (Mitalipov and Wolf, 2014). So, these scientists argue that mitochondria really do not differ much from each other, so mitochondrial heteroplasmy (two types of mitochondria per cell) should not be an issue. Furthermore, the article stresses that the mitochondria gene defects to be treated with MRT “cause severely debilitating and life-threatening conditions in children, and it might be considered unethical to deny MRT gene therapies for these diseases if concerns about safety and efficacy were addressed adequately.” These authors stand by the idea that slight alterations to a family gene line using MRT simply does not match up to the hardship associated with mitochondria diseases in a person’s body. An article issued on behalf of the American Society for Reproductive Medicine presents a plea for mitochondria replacement therapy. Primarily, mitochondria replacement therapy prevents the passing down of a genetic disorder, thus improving the offspring’s individual life, and indirectly, the lives of future members of society. Additionally, physicians have the obligation to act on behalf of their patient’s well-being. If a treatment exists that could potentially improve a patient’s life in a safe way, denying them such a treatment would be unjust (Klitzman et al., 2015). On a further pro-MRT note, several members of Parliament were quoted as saying that MRT is more like “changing a battery pack than about genetic modification” (Parliament.UK, 2015). The parent’s nuclear DNAs would remain unchanged, so does the benefit of eliminating a severe debilitating or fatal human disease override the societal objection to slightly “changing the human germ line”.

Conclusions for Part-4

Perhaps the most counterintuitive aspect of all assisted reproductive technologies is that the offspring have absolutely no influence on any treatment their parents decide to exercise.

Though many cultures require people to have lived a certain number of years before being treated as adult, decision making members of society, the potential effects of assisted reproductive technologies and treatments extend far beyond the minimum number of years required to be considered an adult. Even so, technologies will continue to be developed serving a myriad of patients searching for answers. Louise Brown, the first test tube baby born in 1978, considers the debate of assisted reproductive technologies from a unique perspective. Cole Moreton of *The Independent* conducted an interview with her in 2007. At the time, she had recently given birth to her own child naturally, and without the use of IVF. In the interview, Brown reveals that other kids picked on her in school for being the “test tube” baby. Resultantly, Moreton reports that, “Despite lifelong media attention, Louise has always sought to stay private rather than make money from her fame” (Moreton, 2007). The life of Louise Brown marks an important achievement for science, but now she focuses on raising her own child. One might expect a “test tube baby” to struggle with self-perception issues, but Louise seems to be coping well. Will MRT babies suffer similar taunts at school? Science tells us whether or not we can do a procedure, but ethics tells us whether or not we should do that procedure.

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Part-5: The Legalities of IVF and MRT

Maureen Hester

Thomas Jefferson is attributed as saying, “I consider ethics, as well as religion, as supplements to law in the government of man.” The inextricable relationship between these three primary principles results in a perpetual ambiguity as to delineating between them. And their overlaps are visible in the design of each country’s laws. Due to ethical, and often religion based controversies surrounding the IVF and mitochondrial replacement therapy (MRT) procedures (discussed above), some countries have enacted laws that tightly regulate, or even ban, the treatments. The regulatory oversight of these interesting techniques varies not only from country to country, but even from state to state in America. A review of the current literature on the legalities related to IVF and MRT procedures opens the door to many questions about legal consistencies, and as the science advances it is important to maintain an open discussion and enact laws that keep up with the technology.

IVF procedures have been used in humans since the birth of the first test tube baby, Louise Joy Brown, in England on July 25, 1978 ([BBC News, 1978](#)). Since the first IVF birth, the use of IVF has expanded worldwide with millions of procedures performed. Louise is now 37 years old, and she and her son are closely being watched for any adverse health complications. The world has had 37 years to discuss and enact laws regulating IVF procedures. But despite all this time, the regulation of IVF is highly variable across the globe, with inconsistencies, such as the regulation of IVF surrogates, the semi-commercialism of reproduction, and IVF tourism. Views on the techniques are split within certain nations and religions, and in many cases the laws are unclear. This review of the literature aims to not only clarify the current laws but open a discussion about this interesting technology.

U.S. IVF Laws

Assisted Reproductive Therapy (ART) is one of the most highly regulated of all medical practices in the U.S. ([American Society...2010](#)). It is regulated on 3 levels: state, federal, and professional self-regulation ([Minieri, 2013](#)). The state level involves the licensure and monitoring of qualified physicians who meet minimum standards for skill and education. The federal level of regulation is orchestrated by The Centers for Disease Control and Prevention (CDC), The Food and Drug Administration (FDA), and The Centers for Medicare and Medicaid Service ([American Society...2010](#)). The practices are also self-monitored and regulated by The American Society for Reproductive Medicine (ASRM) and the Society for Reproductive Technology (SRT).

At the federal level, while the FDA does not require screening of the recipients of egg or sperm donations, the ASRM highly recommends it ([Practice Committee... 2013](#)). The U.S. generally allows IVF to be performed for any sexually intimate partners, but the Code of Federal Regulations (CFR) includes some requirements for pre-screening and some restrictions on donations ([21 CFR 1271.90.a.2](#)). Donors must be of legal age (preferably between 21 and 34), and typical donor screening includes testing for HIV, Hepatitis B and C, gonorrhea, chlamydia, syphilis, cystic fibrosis carrier, and mental health screenings ([Practice Committee.. 2013](#)). Oocyte donations will not be accepted in cases where the woman has a blood clotting disorder,

where the woman has been incarcerated within the past 12 months, where the woman has had sexual intercourse with a man who has had intercourse with another man within the past 5 years, the woman has received a small pox vaccine, or if the woman has dementia or any other neuro-degenerative disorder.

There are also restrictions for the use of gestational carriers for both the genetic parents and the surrogate. The ideal gestational carrier is between 21 and 45 years of age, has had at least one term, uncomplicated pregnancy, has had no more than either 5 previous deliveries or 3 caesarian sections, and should have a stable family environment ([Practice Committee...2015](#)). She must pass a multitude of medical screenings as well as undergo a psychological evaluation. Genetic parents will be considered ineligible for the use of a gestational carrier in cases where there is physical evidence of anal intercourse in the male partner, evidence of percutaneous drug use, unexplained jaundice, corneal scarring, or evidence of risk for sexually transmitted infections (STIS) ([Practice Committee..2015](#)).

At the state level, regulations vary from state to state. For example, in 2009, the state of Tennessee proposed a bill that would have defined donor IVF as a type of adoption ([Fiscal Note, 2012](#)), but the bill did not pass ([Legislative Update, 2012](#)). Had the bill passed, it would have increased the variance in laws from state to state, which in the future could be something problematic merging with federal regulations.

IVF Laws in Other Western Countries

In other Westernized and European countries, regulation of ART is just as variable as it is state to state within the US. For example, Costa Rica initially banned the use of IVF technology; their Supreme Court ruled IVF as unconstitutional in that strongly Catholic country because it "violated life" ([IVF Prohibition...2009](#)). Catholicism is the only religious institution to completely ban IVF under any circumstance ([Roberts, 2006](#)). As discussed in the previous Lit Review section, the basis of the Vatican's condemnation of the therapy is due to the argument that (1) IVF can involve destruction of the embryos, i.e., the "destruction of human life," and (2) by engaging in assisted reproduction, humans are "technologically interfering with a process that should remain under God's dominion" ([Roberts, 2006](#)). In 2009, Costa Rica was the only country in the western hemisphere that forbade IVF. But in 2012, in spite of the Costa Rican government opposition to the technique, and strong religious opposition, the IVF ban was overturned by the Inter-American Court of Human Rights on 20 December 2012 ([New York Times, 2012](#)).

Poland is another mainly Catholic country, and this is influence is a major source of their debate. While IVF has been used in Poland for over 25 years, surprisingly no major debates on the topic occurred until 2007. In 2007, the Polish Minister of Health, Ewa Kopacz, decided she would try to finance IVF from the state budget ([Radkowska-Walkowicz, 2014](#)). Health care in Poland is public, but prior to 2007 IVF patients had to resort to treatments from the private sector. With about 20% of the population having trouble conceiving, about 40 private infertility clinics were not regulated by the state ([Radkowska-Walkowicz, 2014](#)). IVF is currently unregulated in Poland, but has been the subject of several drafts of bills, ranging from no limitations on IVF, to IVF punishable by imprisonment. In this country of Catholics, IVF is

often compared to abortion, and is sometimes referred to as "the murder of Polish citizens". Not only do some legislators want IVF criminalized, they also encourage the stigmatism of children conceived through IVF (Radkowska-Walkowicz, 2014).

In Australia, in 2000 a Victorian federal court ruled in the Leesa Meldrum case that a previous ban on IVF for all single infertile women and lesbians was sex discrimination (O'Connor, 2002), allowing the procedure. In 2002, the earlier discrimination conclusion was affirmed when an appeal to the Australian High Court was rejected on procedural grounds. In 2007, Australia's Victorian state government eliminated their restrictions on IVF for single women and lesbians, leaving South Australia as the only state retaining the ban (Hoare, 2007).

The European Union shows a wide variety of laws related to IVF, shown in **Table 1**. As expected, the laws vary widely. For example, Croatia, the Czech Republic, Estonia, Ireland, Latvia, Luxemburg, Malta, Poland, Portugal, Romania, Slovakia, and Slovenia all fully allow access to IVF by anyone, while all other European countries restrict access to specific people.

Table 1: ART in the European Union (Minieri, 2013).

Country	MAR legislation	Access to IVF/ICSI	Gametes donation	Cryopreservation and PGD
Austria	<i>Fortplantungsmedizingesetz</i> (01.07.1992); Tissue safety Law (19.03.2008)	Heterosexual couples only	Sperm donation	Allowed, Forbidden
Belgium	Regulation of IVF Centers Law (15.02.1999); Law on embryo research (11.05.2003); Law on conditions reimbursement laboratory (04.06.2006); Tissue and cell directives (19.12.2008).	Homosexual couples and single women	Allowed	Both Allowed
Bulgaria	MAR is covered by a general health law	Lesbian couples and single women	Embryo donation allowed	Both Allowed
Croatia ²	No specific legislation in place.	Allowed	Sperm donation	Partly allowed, Forbidden
Cyprus	MAR is covered by a general health law	Homosexual couples and single women	Allowed	Both Allowed
Czech Republic	No data could be obtained on the existence of MAR- specific legislation	No restrictions mentioned	Post-mortem use of gametes partly allowed	Forbidden, Allowed
Denmark	Statute no. 923 of 2006, 284 of 2007, 534 of 2008	Homosexual couples and single women too	No embryo donation (only for research)	Partly allowed, Allowed
Estonia	Assisted fertilization and Protection of the Embryo Law	Allowed	Allowed	Allowed, Forbidden

Country	MAR legislation	Access to IVF/ICSI	Gametes donation	Cryopreservation and PGD
Finland	Act of Assisted Reproduction (22.12.2006); Act of Medical use of human organs, tissues and cells (02.02.2001)	Homosexual couples and single women too	No embryo donation. Identifying info	Both Allowed
France	Statute no. 800 of 2004	Married or co-habiting couples at least for two years	No embryo donation	Allowed, Partly allowed
Germany	Gesetz zum Schutze von Embryonen (13.12.1990); Gesetz über Qualität und Sicherheit von menschlichen Geweben und Zellen (20.07.2007)	Heterosexual married or co-habiting couples only	Sperm donation for IUI only. Identifying info	Both partly allowed
Greece	Statute no. 3305 of 2005	Heterosexual couples and single women	Embryo donation allowed	Both Allowed
Hungary	MAR is covered by a general health law	Heterosexual couples and single women	Allowed	Both Allowed
Ireland	MAR is covered by a general health law	Allowed	Allowed	Both Allowed
Italy	Statute no. 40 of 2004	Heterosexual married or co-habiting couples only	Forbidden	Both Partly Allowed
Latvia	MAR is covered by a general health law	Allowed	Allowed	Both Allowed
Lithuania	MAR is covered by a general health law	Heterosexual couples only	Forbidden	Forbidden, Allowed
Luxembourg	MAR is covered by a general health law	Allowed	Allowed	No legislation
Malta	MAR is covered by a general health law	Allowed	Allowed	Both Forbidden
Netherlands	Act on In Vitro Fertilization (01.04.1998); Embryo Act (20.06.2002); Law on data from donors for artificial reproduction (25.04.2002); Law on safety and quality of human tissues (06.02.2003).	Homosexual couples and single women too	Post-mortem use of gametes allowed. Identifying info	Both Allowed
Poland	MAR is covered by a general health law	Allowed	Allowed	Both Allowed
Portugal	Statute no. 32 of 2006	Allowed	Allowed	Allowed, Partly allowed
Romania	No specific legislation in place, only statute no. 95 of 2006 based on Cell and Tissue Directive	Allowed	Allowed	Both Allowed

Country	MAR legislation	Access to IVF/ICSI	Gametes donation	Cryopreservation and PGD
Slovakia	No data could be obtained on the existence of MAR- specific legislation	Allowed	Allowed. Identifying info.	Both Allowed
Slovenia	Act on infertility treatment and procedures of biomedical assisted procreation (28.07.2000); Act for quality and safety of tissues and cells (02.07.2007)	Allowed	Forbidden	Forbidden, Allowed
Spain	Royal Decree no. 1301 of 2006	Lesbian couples and single women	Post-mortem use of gametes allowed	Both Allowed
Sweden	Statute no. 286 of 2008	Married or co-habiting couples only	Allowed. No Embryo donation. Identifying info	Allowed, Partly allowed
United Kingdom	The Human Fertilisation and Embryology Act 1990, 2008; The Human Reproductive Cloning Act 2001.	Married or co-habiting couples and single women	Allowed. Identifying info	Allowed, Partly allowed

The use of pre-implantation genetic diagnosis (PGD) combines molecular genetics and ART to screen embryos for genetic diseases (Zahraa, 2006). Usually, a single cell is removed from a cleavage-stage IVF embryo and analyzed by polymerase chain reaction (PCR) to determine whether that embryo's DNA has the genetic mutation. If not, it is implanted into the uterus. This screening enables the selection and implantation of healthy embryos. In 1997, the European Society for Human Reproduction and Embryology PGD Consortium was established

to monitor PGD treatments and their outcomes (Gianaroli, 2014). Italy allows medically assisted conception only for infertile couples, not for genetic testing, so in that country couples with known genetic diseases cannot screen in advance for it (Gianaroli, 2014).

IVF Regulations in Islam

Islam, a monotheistic religion distinguished by the following of the Qu'ran, can be divided into two major denominations: Sunni Islam and Shia Islam. Sunni Muslims comprise 80% to 90% of the world's Muslim population today (Inhorn, 2006). Within Islam, marriage is considered a dutiful form of worship, and the second function of this union is procreation. Preservation of heredity is very important to Muslims, and therefore IVF is generally accepted and allowed within a marriage, so long as the couple's genetic lineage is maintained (Alghrani, 2013). The acceptable ways in which IVF can be practiced, however, differs between the two denominations. With respect to Islam, Sunni Muslim nations generally allow IVF if performed on married couples using their own sperm and eggs, but do not allow the use of *donor* eggs (Inhorn, 2011). It is the belief of Sunni Muslims that gamete donation is detrimental to both the child as well as society because it compromises sexual decency and lends itself to the risk of half sibling incest in the future, and the donated gamete would go against the principle of marriage as the only framework for procreation (Ishak, 2014). Any case in which the conception of the child could mirror adultery or lead to a confused lineage is prohibited (Zahraa, 2006). This means that the sperm and eggs must be taken from the intended parents, and the egg must be placed in the womb of the mother.

Shi'ite Muslims are less conservative than their Sunni counterparts when it comes to reproductive technology. In Shi'a Muslim Iran, interestingly, the country bans sperm donation while allowing egg donation (either fertilized or unfertilized). In the late 1990s, the Supreme Jurisprudent of a Shi'a branch of Islam issued a Fatwa which permitted the use of donor technologies, stating in the case of egg donation that both the donor and the infertile mother must abide by the religious codes of parenting. The resultant child may inherit from the genetic mother, and the infertile mother is considered an adoptive mother (Inhorn, 2006). In Shi'a communities, fertilized embryos can be donated from married couples to other married couples for the sake of IVF, while unfertilized eggs can be donated to another married couple in the context of *mut'ah* or temporary marriage to the [donor] father (Inhorn, 2011). In the past, *mut'ah* was often used for widows in need of financial security, but it is now being invoked to make egg donation legal within a Shi'a marriage. In 2003, the Iranian parliament passed a law restricting gamete donation to married persons; egg donation is allowed if the husband marries the donor temporarily, but sperm donations are illegal as a sperm donor cannot temporarily marry an already married woman. While not yet widely accepted, embryo donation is allowed as long as donated from one married couple to another married couple who would be treated as adoptive parents (Inhorn, 2006).

IVF Regulations in Eastern Countries

Despite their relative proximity to each other, Eastern countries enforce very different ART regulations. Some Eastern countries believe that reproduction is tightly coupled with

marriage, so have enacted laws that ban individuals from IVF if they are not married. Prohibited individuals might include single females, lesbians, or surrogates. For example, in 2003, government agencies in China banned the use of IVF procedures by unmarried women or by couples with specific types of infectious diseases ([Redorbit.com, 2003](#)), however, the Chinese Ministry of Health has approved IVF facilities for infertile married couples that wish to have children ([Flippova, 2010](#)). In addition to who is allowed to receive IVF, the way it is performed is also regulated. In 2007, Taiwan with its Human Reproduction Law limited the number of embryos that can be transferred to no more than four ([Wu, 2012](#)). Of the 43 countries that regulate embryo transfer numbers, Taiwan is considered to be the most lenient. Taiwan's first ART regulation stated that it be made available to infertile married couples, and that sperm and eggs could not be commoditized ([Wu, 2012](#)). Nearby Japan is considered one of the world's most advanced countries in terms of non-donor IVF with over 600 registered infertility clinics ([Shimazono, 2013](#)). Infertility seems to be increasingly prevalent in Japan, and it could be attributed to a tendency to marry late, which subsequently could lead to an increasing demand in donor-oocytes ([Shimazono, 2013](#)). While at the time there are no legal statutes, The Japanese Society of Obstetrics and Gynecology recommends refraining from the use of third party gametes. The Japanese Society for Reproductive Medicine issued a statement saying that under limited circumstances egg donations by a sibling or family member could be acceptable ([Shimazono, 2013](#)). In more lenient Russia, gestational surrogacy is completely legal, and it is available for all adults who wish to become parents ([Lysytsia, 2011](#)). Surrogacy is regulated by the Family Code of Russia, and no court consent or adoption is required even in cases where the intended parents will have no genetic relation to the child. But surrogate children of unmarried or single Russian persons must be registered with the court ([Lysytsia, 2011](#)).

Special Laws for Surrogacy

Surrogacy, the use of a woman volunteer to carry an embryo, can be divided into two classes based on the source of the genetic material: 1) traditional surrogacy, and 2) gestational surrogacy. Traditional surrogacy involves the artificial insemination of a woman with the sperm from the male of the couple in need of a surrogate, making him the father and the inseminated woman is both the genetic and gestational mother. In gestational surrogacy, eggs and sperm are taken from the donors, fertilized, and implanted into the surrogate, who has no genetic tie to the baby ([Sharma, 2006](#)). Gestational surrogacy is the most commonly used type of surrogacy today, and it eliminates a genetic tie between the child and surrogate ([Gabry, 2012](#)).

The use of surrogates is especially controversial in some countries. Several countries have outright banned the use of surrogates, including: Argentina, Australia, Austria, Austria, Canada, China, Costa Rica, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Saudi Arabia, United Kingdom ([IVF Prohibition...2009](#)). Other countries have enacted laws that allow the use of surrogates, but only under specific conditions. These countries include Belgium, Brazil, Finland, Georgia, Greece, Holland, Hungary, India (no laws), Ireland, Israel, Korea, Mexico, Norway, Russia, South Africa, Ukraine, and the USA ([IVF Prohibition...2009](#)).

In the United States, most states do not have specific provisions addressing surrogacy, but even when present, they are enforced inconsistently ([Gabry, 2012](#)). New York, Washington D.C., and Michigan have all completely outlawed surrogacy ([Gabry, 2012](#)). In states such as

Louisiana and Nebraska, surrogacy contracts are considered “contrary to public policy” and are therefore unenforceable, but even these bans are unclear, for example, in Louisiana where only traditional surrogacy is addressed (Gabry, 2012). In North Dakota, traditional surrogacy is banned, and any contracts involving traditional surrogacy are considered void by law. In the case of gestational surrogacy, the law is clear in stating that the resultant child of a gestational surrogacy is the child of the intended parents (Gabry, 2012). In Florida, surrogacy arrangements are considered pre-planned adoptions by the law. However, limitations on the use of surrogates in Florida are extensive: at least one of the intended parents must be genetically related to the child, the parents of the intended child must be married (which eliminates the ability of same sex couples to utilize a surrogate), and the mother must have a health condition which makes her unable to carry the child herself (Gabry, 2012). States such as New Hampshire, Virginia, and Utah require a judicial preauthorization for a surrogacy contract to be enforceable (Pelzman, 2013).

Based on the wide range of surrogacy laws in the U.S., it is not hard to imagine the range of surrogacy laws around the world. Countries such as Ireland, Denmark, and Belgium allow surrogacy only when the mother is not paid, while countries such as India, Russia, and the Ukraine allow commercial surrogacy (BBC News, 2014). Legislation regarding surrogacy in Thailand is unclear, but bills are being drafted that would require surrogates to be blood relatives of the intended parents (BBC News, 2014). Not only has Australia completely banned surrogacy, but it has criminalized the act of traveling and participating in commercial surrogacy (BBC News, 2014). The lack of consistency and clarity in the regulation of surrogacy foreshadows similar problems in the future regulation of MRT.

Fertility Tourism Laws

The ban on IVF for individuals in some countries has given rise to “fertility tourism”, where an individual travels to other another country to undergo the procedure. Different ART regulations in different areas of the world have created "reproductive borders." The borders have been created by national legislations affecting the high cost of ART, waiting lists, and limitations such as reproductive age or sexual orientation, and the borders are being crossed to circumvent these complications and expedite the process of having a child (Bergmann, 2011). A statistical breakdown of the reasons residents inside the European Union cross borders are illustrated in **Table 2** below (Minieri, 2013). For example, in Italy 70.6% of the respondents sought IVF in other countries due to legal bans within Italy, and 43.3% wanted better quality care. This same trend is reflected in the other European countries in the table.

Table 2: Reasons for Crossing Borders in Relation to Reproductive Tourism (Minieri, 2013)

Country of residence	Legal Reasons	Access Difficulty	Better Quality	Previous Failure
Italy	70.6	2.6	46.3	26.1
Germany	80.2	6.8	32.8	43.5
France	64.5	12.1	20.6	18.7
The Netherland	32.2	7.4	53.0	25.5
UK	9.4	34.0	28.3	37.7
Sweden	56.6	13.2	24.5	5.7
Total %	54.8	7.0	43.2	29.1

Within the U.S., because no current laws prohibit international surrogacy, the outsourcing of gestational surrogates to Indian women by U.S. citizens is becoming more commonplace for American couples hoping to have a child (Stephenson, 2009). In addition to the varying surrogacy laws in the U.S., cost is a major factor for those seeking these services internationally. The cost of using a surrogate (in 2009) in India averaged \$20,000 while the same process in the U.S. can cost \$90,000 (Stephenson, 2009). Other countries with strict IVF laws leave IVF tourism as the only option for some couples. In accordance with Germany's Embryo Protection Act of 1990, egg donation is strictly forbidden by German Law; sperm donation, however, is not considered something that can interfere with kinship and is therefore legal and acceptable (Bergmann, 2011). For German couples, both Spain and the Czech Republic have become an oasis of fertility treatments. The two countries have been coined "the most important destinations for egg donation inside The European Union," (Bergmann, 2011), for reasons which include their vast tourism business, technological amenities, and the anonymity of egg donations. This anonymity also draws couples from the Denmark, Sweden, and surprisingly even from the U.K. where ART laws are not very restrictive. Aside from the desirable anonymity, shortages of gametes in the area is another factor which encourages travel (Hudson, 2011). A similar shortage of donor oocytes in Japan has led to an increase in couples seeking donations from other Asian countries (Shimazono, 2013). This trend of many couples being willing to travel to receive medical treatments unavailable to them in their home countries will likely be applicable to those in search of MRT.

U.K. MRT Laws

Mitochondrial Replacement Therapy (MRT) is a form of IVF designed to aid in the conception and development of a healthy child for women who suffer from mitochondrial disorders. The controversy surrounding the use of ART and IVF seemingly intensifies as the technology advances, and the manipulation of mitochondrial DNA (mtDNA) adds a new dimension to the debate.

The U.K has been actively engaged in MRT debates for over 10 years now, and in 2015 was the first country to finally approve the procedure. The progress and considerations of U.K. MRT laws are summarized in **Table 3**.

Table 3: MRT Regulations in the U.K. (HFEA Review, 2014)

Date	Consideration
2005	Research licence for pronuclear transfer granted.
May 2010	The Authority's Scientific and Clinical Advances Advisory Committee considers research developments.
February 2011	The Secretary of State for Health asks the HFEA to carry out a scientific review to scope "expert views on the effectiveness and safety of mitochondrial transfer".
April 2011	<p>The panel of experts, co-ordinated by the HFEA, reports to the Secretary of State for Health on the safety and efficacy of methods to avoid mitochondrial disease.</p> <p>Key findings include:</p> <ul style="list-style-type: none"> • Preimplantation genetic diagnosis (PGD) can only reduce, not eliminate, the risk of transmitting abnormal mitochondrial DNA (mtDNA) leading to mitochondrial disease. • The panel concluded that the techniques of maternal spindle transfer (MST) and pronuclear transfer (PNT) are potentially useful for a specific and defined group of patients whose offspring may have severe or lethal genetic disease, due to mutations in mtDNA, and who have no other option of having their own genetic child. • A number of recommendations for further work it wished to see before a decision was made to move to treatment, including a proposed set of experiments that it felt to be critical and a number of recommended experiments that would be beneficial; both sets of recommendations can be found at Annex C of this report and at Sections 5.4 and 5.5 of the 2011 report⁶.
June 2011	The Authority's Ethics and Law Committee considers ethical issues.
January 2012	The Secretary of State for Health and the Secretary of State for Business, Innovation and Skills ask the HFEA to carry out public dialogue work on the ethics and public attitudes towards mitochondrial replacement.
January 2012 – August 2012	Public dialogue and consultation work planning and preparation. Public dialogue work takes place (deliberative public workshops and public representative survey took place).
September 2012 – December 2012	Open consultation runs (open consultation questionnaire, open consultation meetings and patient focus group).

December 2012	The Secretary of State for Health asks the HFEA to provide an updated view of the science to support the assessment of the efficacy and safety of MST and PNT.
January 2013	<p>The panel of experts reconvened and call for evidence issued. Key findings include:</p> <ul style="list-style-type: none"> • The panel's view remained as it was in 2011: that MST and PNT have the potential to be used for all patients with mtDNA disorders, which may make them preferential to PGD in the future. In patients with homoplasmy (all containing mutant mtDNA) or high levels of heteroplasmy (partially containing mutant mtDNA), these are the only techniques that would make it possible for them to have a genetically related unaffected child. • The panel was of the view that there was more published work available to support MST than PNT, but there was still insufficient evidence to recommend one transfer technique over the other. • Once assessed as safe to use in clinical practice, the panel strongly recommended that permission should be sought from the parents of the children born from MST or PNT to allow them to be followed up for an extensive period (and that permission should then be sought from the children themselves, when old enough). The panel recommended that any female born following MST or PST is advised, should she wish to have children of her own, that her oocytes (eggs) or early embryos are analysed by PGD in order to select for embryos free of abnormal mtDNA. This has the potential to eliminate risk in subsequent generations. • The 2013 panel continued to recommend the set of minimum critical experiments first outlined in the 2011 report. However they highlighted that the recommended work in understanding MST using fertilised oocytes and PNT using normally fertilised oocytes was underway and noted that progress was good. The panel's comments on the progress made on the recommended research are summarised in Annex C of this report and in Sections 2.3 and 3 of the 2013 report⁷. • Further studies on mosaicism in human morulae (comparing individual blastomeres) and on human embryonic stem (ES) cells (and their differentiated derivatives) derived from blastocysts, where the embryos have (i) originated from oocytes heteroplasmic for mtDNA and (ii) been created through MST and PNT using oocytes or zygotes with two different variants of mtDNA. Although experiments are already reported on embryonic stem (ES) cells and their derivatives with MST, further corroborative experiments
	<p>would be valuable.</p> <ul style="list-style-type: none"> • A recommendation made by the panel in 2011 to carry out PNT in non-human primate models was considered, in the light of new evidence, to be both difficult and unnecessary. Such experiments were therefore no longer mandatory.
March 2013	The findings of the public dialogue and the 2013 scientific review update were submitted to Government, together with considerations of how the techniques might be regulated. The public dialogue work concluded that the public were generally supportive of these techniques, although concerns around safety, the donor role and the regulation of the techniques were highlighted.
June 2013	The Government announced that, based on the findings of the HFEA's public dialogue and consultation exercise and the views of the panel, it would move forward with draft regulations for public consultation.
February 2014	The Department of Health opened a consultation on draft regulations for the use of these techniques to prevent mothers passing on serious mitochondrial diseases to their children. Alongside this the HFEA was asked to provide a further updated view on the science.

In 1990, Britain's Parliament approved the Human Fertilization of Embryology Act of 1990 ([Gov.UK, 1990](#)). The 1990 act prevented any human nuclear transfer trials (which would include MRT), and at the same time it established Britain's fertility regulator, the Human Fertilization and Embryology Authority (HFEA). Among its other responsibilities, the HFEA licenses and monitors all human embryo research performed in the U.K., and these responsibilities continue to this day. In 2005, the HFEA issued a research license to the Newcastle Centre for Mitochondrial research to "investigate the feasibility of using IVF-based techniques to prevent the transmission of mitochondrial disease" ([HFEA.gov, 2005](#)). But in that year, MRT clinical trials were still prohibited by the 1990 act, so in 2008, Parliament amended the 1990 Act to allow HFEA to license and monitor MRT clinical trials, creating the Human Fertilization and Embryology Act 2008 ([Gov.UK, 2008](#)).

In 2011, the HFEA convened an expert panel to review the effectiveness and safety of MRT ([HFEA Review, 2011](#)). The panel concluded in their report entitled "Scientific Review of the Safety and Efficacy of Methods to Avoid Mitochondrial Disease Through Assisted Conception" that there was no scientific evidence showing that MRT is unsafe ([HFEA Review, 2011](#)). Their updated reports in 2013 and 2014 concluded the same. In 2012, the HFEA initiated a public consultation process on the social and ethical implications of MRT. The outcome, published in 2013 ([HFEA Advice to Government, 2013](#)), was a general support for permitting MRT so long as it is safe and done within a regulatory framework. On February 3, 2015, the House of Commons voted to enact an amendment to the earlier "Human Fertilization and Embryology Act 2008" which allowed MRT clinical trials. The 2015 amendment would allow MRT to the public. Two techniques were discussed: maternal spindle transfer (before fertilization) and pronuclear transfer (after fertilization but before nuclear fusion) ([Parliament.UK, 2015](#)). In this debate, the analogy of changing the battery pack on a camera was used to illustrate the concept of MRT for lawmakers - it does not change the camera; it just allows the camera to function better. The important clarification between nuclear DNA and mtDNA was also made to the House of Commons at this meeting. Opposition to MRT was expressed in terms of concern over alteration of the embryo's germline and corresponding consequences, "once this alteration has taken place and once the genie is out of the bottle, and once these procedures that we are being asked to authorize today go ahead, there will be no going back for society, and certainly not for the individuals concerned" ([Parliament.UK, 2015](#)). The amendment was approved in the House of Commons by a vote of 382 in favor, and 128 against ([Callaway, 2015](#)).

Once the 2015 MRT amendment was approved in the House of Commons, it moved to the House of Lords, which voted to approve the measure on February 24, 2015 ([Vogel, 2015](#)). On that day, the House of Lords initially debated for several hours on a proposed amendment that would have established a committee to further study the possible risks of MRT, but voted to defeat that amendment and quickly approved the original MRT amendment. The 2015 approval votes in the House of Commons and House of Lords makes the U.K. the first country in the world to explicitly allow MRT for the public. The goal of the legislation is to prevent the transmission of diseases caused by mutations in mtDNA. But passage of the amendment allowing MRT to the public is not the end of the U.K.'s legal oversight. The amendment gives Britain's fertility regulator, the HFEA, authorization to begin issuing licenses to facilities to perform the technique. Researchers who wish to offer the MRT service to couples must apply for and receive a license from HFEA ([Vogel, 2015](#)). However, before granting licenses, the HFEA could require further evidence that the MRT procedure is safe, so it may authorize scientific

studies to acquire more data. And in any case, the HFEA will likely carefully consider applications on a case-by-case basis prior to issuing any license (Callaway, 2015).

Policy makers in the UK addressed several key points that will be of interest to the U.S. debate. The first is they approved MRT only in cases involving mitochondrial diseases, and the technology cannot be used for women who are becoming too old to reproduce, and cannot be used to create "designer babies" (Klitzman et al., 2015). Although some groups in the U.K. objected to the MRT manipulation of the germline, the Medical Research Council and Wellcome Trust of the United Kingdom decided that MRT will not have an effect on the characteristics associated with an individual's identity (Reinhardt, 2013). Second, the UK law indicates that the mitochondrial donors will be viewed as organ donors in the eyes of the law, and will have no legal parental claims to the resultant child (Klitzman et al., 2015).

U.S. MRT Laws

The U.S., unlike the U.K.'s HFEA, lacks a single governmental agency for regulating reproductive technologies. For MRT, jurisdiction has been claimed by the FDA, especially their Office of Cellular, Tissue, and Gene Therapies of the Center for Biologics Evaluation and Research (FDA.gov, 2001). So, the FDA currently has the power to regulate MRT as a form of gene therapy. The jurisdiction of MRT clinical trials is given to the FDA because it involves the manipulation of the germ line (Mitochondrial...2014). Under the FDA's regulations, approval of MRT will require the performance of phase I, II, and III clinical trials using the usual Investigational New Drug (IND) application method for approval to begin any studies. Since 2001, the FDA has enforced a moratorium on performing any human MRT experiments, in part due to the 2001 experiments performed at a New Jersey fertility clinic where healthy mitochondria from a donor egg were injected into a mother's egg to improve fertility (Callaway, 2015). In the late 1990s, 30 babies were born from women who repeatedly suffered from IVF failure and were treated using ooplasm transfer before it was banned (Klitzman et al., 2015).

To date, the FDA has not officially considered or approved any MRT human clinical trials. On July 2, 2013, the FDA published their Draft Guidance for Industry: Considerations for the Design of Early Phase Clinical Trials of Cellular and Gene Therapy Products (Federal Register, 2013), but that draft guidance did not mention MRT.

On February 25 and 26, 2014, the FDA convened a 2-day meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee to discuss "oocyte modification in assisted reproduction for the prevention of transmission of mitochondrial disease" (FDA.gov, 2014). This committee included doctors, researchers, representatives of industry, and representatives of patient groups (Vogel, 2014). Although this 2014 meeting represented the first time the U.S. had officially discussed the MRT technique, there was no conclusion from the meeting. Several panelists went on record as saying there was "probably not enough data in animals to move to clinical trials without answering a few additional questions" (Begley, 2014). Some of the additional data the scientists wanted to see were the long-term health of monkeys conceived through MRT (Callaway, 2015). Evan Snyder, a stem cell biologist at Sandford-Burnham Medical Research Institute (La Jolla, CA), Chair of the FDA panel, said "It will probably take two to five years to fill in these [data] gaps" (Callaway, 2015). Overall, the committee agreed

that the following areas needed to be explored: 1) the interaction between mutant and wild-type mitochondria; 2) the effect of mitochondrial genotypes in specific cell types; 3) the induction of apoptosis by mutant mitochondria; and 4) the interaction of different mitochondrial genotypes in different tissues ([FDA.gov, 2014](#)). Standardization of mitochondrial manipulation techniques was also recommended. The discussion of eventual human clinical trials indicated that, "for trials to prevent transmission of mitochondrial diseases, eligibility criteria might limit enrollment to women with specific mtDNA mutations that are known to yield consistently and stereotypically the most dire disease states, clinical manifestations, disease severity, extent of heteroplasmy, or other factors. Selection of only male embryos for transfer might be an option to minimize the risk of transmitting mitochondrial disease to subsequent generations" ([FDA.gov, 2014](#)). There would need to be a significant monitoring of any human clinical trials, and this meeting started a constructive dialogue in the ways this could and should be done.

In September 2014, the FDA commissioned an Ad Hoc Committee of the Institute of Medicine (IOM) to consider the "ethical and social policy considerations of novel techniques for the prevention of maternal transmission of mitochondrial DNA diseases" ([National Academies, 2014](#)). Two of their discussion sessions will be open to the public ([Cohen et al., 2015](#)). Until the release of IOM's report (estimated to be about April of 2016, 19 months after the September 2014 start date), no further FDA actions on MRT are expected. In the meantime, any IND applications for MRT human clinical trials will remain on hold.

On June 17, 2015, a draft spending bill was released in the House of Representatives that if passed would restrict any research on heritable genetic changes to human embryos. If the bill passes, it would ban the FDA from reviewing such research, and might include the technique of MRT. Representative Andy Harris (R-MD) commented on the bill saying "it would slow down the FDA's review, allowing everyone a chance to review the ethics", while Henry Miller, a physician and biologist at Stanford University said "it would set a new standard for congressional stupidity and inhumanity" ([News in Brief, 2015](#)). The bill is expected to be voted on in July.

Dr. Shoukhrat Mitalipov, a biologist at the Oregon Health and Science University (Portland, OR), who has performed MRT experiments on monkeys, and who hopes to apply for FDA approval to perform human MRT, stated "Although [the US] regulatory debate is a bit behind the United Kingdom's, the US is going down the same path" ([Callaway, 2015](#)). Australia is also considering allowing human MRT, but David Thorburn, a geneticist at the University of Melbourne said "My gut feeling is that [MRT is] unlikely to succeed [in Australia] until this has been done in practice in the U.K." ([Callaway, 2015](#)).

Part-5 Conclusions, Problems, and Questions

Advances in science and technology are perpetual instigators of endless debates worldwide. The issues which arise with these advances range from their morality, to the ways they are regulated, to how they mesh with different religions, to the extent to which the technology should be applied. Now that a global discussion about Mitochondrial Replacement Therapy has begun, one can only hope that the dialogue will remain open and productive, and eventually help save lives. Many of the same regulatory issues facing IVF technologies will plague MRT, and cohesive regulatory systems will become very important as research continues.

While researching the literature concerning the laws regulating IVF and MRT, several problems were identified that likely will pertain to MRT in the U.S., and will be the focus of interview questions performed for this IQP project:

1. The U.K. Debate: With their 2015 approval of MRT, the U.K. has led the way with debates and legislation with this new technology. The U.K. debates focused on three key points that likely will also need to be debated in the US:
 - a. Germline Alteration. Some individuals argue that MRT will allow the creation of designer babies (eugenics). But mitochondrial DNA is not known to control any major outward phenotypes of an individual's identity, and mtDNA represents only about 0.1% of an individual's total genome. Do you think eugenics would be a problem here, and if so, can't we just ban it in any U.S. legislation, restricting MRT only for severe mitochondrial diseases?
 - b. MRT to Treat Age-Related Infertility. The U.K. banned the use of MRT simply to treat age-related infertility, restricting it only for mitochondrial diseases. Do you agree the U.S. should follow suit?
 - c. Parental Rights. It is our understanding that the U.K. legislation gives full parental rights to the genetic mother and father (who provided the nuclear DNA during MRT), but not to the egg donor (who provided the healthy mitochondria). Do you think this point should be addressed in the U.S. legislation?
2. Current Status of the U.S. MRT Debate: It is our understanding that the U.S. likely is not expecting any formal government report on the safety of MRT until about April of 2016, which is the approximate release time of the FDA's report of the Ad Hoc Committee of the Institute of Medicine (IOM). The IOC was charged with considering the "ethical and social policy considerations of novel techniques for the prevention of maternal transmission of mitochondrial DNA diseases". Do you agree that the next regulatory hurdle for MRT in the U.S. is for the technique to be declared safe by this FDA-mandated committee?
3. Reproductive Tourism: Now that MRT has been legally approved in the U.K., will their oversight committee (HFEA) allow couples from other countries to travel to the U.K. to undergo MRT?
4. Which Diseases? Which mitochondrial diseases do you think should be of highest priority for MRT?
5. MRT Stigmatizing: Some early IVF children were taunted in school for being different. Do you think this is still a problem, or now that millions have undergone IVF is this less of a problem? Do you think MRT children will undergo stigmatizing in school?
6. Male Embryo Transfers: Some scientists have suggested enacting laws that restrict MRT to allow transfer only male MRT embryos. Do you think this would actually decrease the chances of any side effects from MRT, because male embryos will still have the same levels of heteroplasmy (1%) as female embryos, and males still suffer from mitochondrial diseases?

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METHODS

To accomplish objective-1, we performed a review of the current literature, including reputable academic journal articles, relevant books, scholarly websites, and other pertinent materials.

To accomplish objective-2, we conducted a set of interviews with various academic researchers, bio-ethicists and legal experts to determine their full range of opinions on the strengths and weaknesses of this new MRT technology, and to determine which obstacles remain for approving MRT in the U.S.

Who: The stakeholders included individuals working with mitochondrial disease patients, individuals performing IVF or MRT procedures, bioethics experts, and MRT legal experts. Some of the stakeholders initially were identified by referral from the project advisor, Dr. David Adams, but other interviewees were identified from the literature as authors on key scientific papers, or by referral from the initial interviewees.

Where and When: Whenever possible, interviews were conducted in person, but the majority were performed by email, phone, or Skype.

How: We developed our interview questions based on our background research. A preliminary set of questions is shown in the Appendix. Based on our background search of each interviewee, we designed a pertinent initial question. Any subsequent questions were based on their response to the initial question. The appendix shows the topics covered in our interviews.

With respect to the method of the interview, after establishing contact with an interviewee, we informed the interviewee about the purpose of our project, and asked for permission to quote them (see interview preamble in the Appendix). If the need arose for confidentiality, we protected it by either not quoting them directly, or by giving them the right to review any quotations used in the final published report, explaining that the interview is voluntary, and explaining that they may stop the interview at any time or refuse to answer any question. At the end of the interview, we sometimes asked the interviewee to recommend other potential stakeholders we might interview, to further increase the number of interviews with key individuals.

With respect to the total number of interviews performed for our project, we discontinued our interviews once we had obtained sufficient information to represent all sides of the MRT problem, and when the unclear points had been clarified.

To accomplish objectives-3 and 4, the IQP team synthesized all of the information collected in our literature research, interviews, and follow-up interviews to ascertain the strength of the evidence for and against MRT, and created recommendations for further research.

RESULTS / FINDINGS

Mitochondrial Diseases Daniela Barbery

This area of the IQP focused on introducing the reader to mitochondria, their functions, and their diseases. Our review of the literature in this area resulted in a several questions for interviewees who work with mitochondrial patients, including having them help us define which diseases are most likely to be treated with MRT, whether MRT would improve patients containing nuclear mutations encoding mitochondrial proteins, and whether another technique besides MRT could cure mitochondrial patients or their offspring.

To investigate these issues further, interviews were performed with several experts on mitochondrial diseases. The first interview was conducted with Dr. Salvatore DiMauro, MD, of the Laboratory of Personalized Genomic Medicine, Department of Pathology and Cell Biology, Columbia University Medical Center (New York, NY). Dr. DiMauro is an expert on mitochondrial diseases, and was an author of a 2005 review article in the *Annals of Medicine*, 37: 222-232, entitled “Mitochondrial DNA and Disease”. When asked his opinion on which mitochondrial disease would best benefit from MRT, he responded “mitochondrial replacement therapy would be the ideal solution for treating mtDNA mutations”. So, Dr. DiMauro seems to indicate that all types of mitochondrial diseases would benefit from MRT, none with higher priority than another. He also did not point out any better technique for treating these diseases.

We next interviewed Dr. Josef Finsterer, MD, PhD, of the Krankenanstalt Rudolfstiftung, Postfach 20, AT-1180 (Vienna, Austria). Dr. Finsterer was sole author on a 2007 review paper published in *Acta Haematologica*, 118(2): 88-98, entitled “Hematological Manifestations of Primary Mitochondrial Disorders”. In this review, Dr. Finsterer concluded that mitochondrial disorders (MID’s) are frequently caused by a single “organic problem” (such as a mutation), but they manifest in multiple systems. The organs and tissues affected most often are the cerebrum, peripheral nerves, and skeletal muscle (all that use high amounts of energy, and would be most affected if energy production is weakened). The review article focused on the hematological manifestations of mitochondrial disorders, including various types of anemias, leukopenia, neutropenia, thrombocytopenia, and pancytopenia. Mitochondrial diseases that mostly manifest in the blood include Pearson’s syndrome (pancytopenia), Kearns-Sayre syndrome (anemia), Barth syndrome (neutropenia), Sideroblastic anemia syndrome. Anemia can also be found as a less predominant symptom in other mitochondrial disorders such as Leigh’s syndrome, MERRF Syndrome, Leber’s Hereditary Optic Neuropathy (LHON), and Friedreich’s Ataxia Anemia. When asked his opinion on which types of mitochondrial disorders are likely to be the first successfully treated by mitochondrial replacement therapy, he replied “all mtDNA disorders may profit from MRT”, and reminded us that the patient would not benefit from the disease, but his/her offspring. However, this interview took place prior to the publication of the August 2015 article showing that stem cells prepared from mitochondrial disease patients, in theory, could be used to treat the patient as well as the offspring.

With respect to whether an alternative approach might be used to treat mitochondrial disorders, we interviewed Dr. Gerald I. Shulman, MD, PhD, of the Howard Hughes Medical Institute, Yale University School of Medicine (New Haven, CT). Dr. Shulman was corresponding author on a 2004 paper in the *New England Journal of Medicine*, 350: 664-671, entitled “Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes”. This is an interesting article that investigated a possible mechanism for why insulin resistance (type 2 diabetes) in the parents is the best predictor of insulin resistance in the offspring. The authors show that insulin resistance in the skeletal muscle of the offspring is associated with dysregulation of “intra-myocellular” fatty acid metabolism located in the mitochondria, so perhaps the insulin resistance in the offspring results from an inherited defect in mitochondrial oxidative phosphorylation. When asked whether this intra-myocellular fatty acid metabolism disease located in the mitochondria (offspring) would be treatable by MRT, he replied “I do not think that simply increasing the number of mitochondria will fix this particular problem (we have already demonstrated this in PGC-1-alpha muscle specific transgenic mice). Instead, I think the solution is to [fix] subtle hepatic mitochondrial inefficiency by promoting increased hepatic fat oxidation and decreased hepatic DAG-nPKC activation (to create improved insulin signaling), and to decrease hepatic acetyl CoA content (decreased pyruvate carboxylase activity/decreased hepatic gluconeogenesis) to decrease fasting plasma glucose concentrations. So, this useful response showed an alternative approach for treating some types of mitochondrial diseases, not by using MRT but by using small molecule protonophores to induce changes in mitochondrial metabolism to improve mitochondrial inefficiency. In his particular case, his lab promotes increased hepatic fat oxidation and decreased DAG activation to improve insulin signaling. This ionophore technique might work in the patients themselves, different than MRT for helping the offspring.

In some cases, modern proteomics techniques have been applied to mitochondrial disease cells for comparison of the total protein profiles to normal cells. We interviewed Dr. Patcharee Lertrit of the Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand). Dr. Lertrit was one of two corresponding authors on a 2014 paper published in *PLoS One*, 9(9): e106779, entitled “Profiling the Mitochondrial Proteome of Leber’s Hereditary Optic Neuropathy (LHON) in Thailand: Down-regulation of Bioenergetics and Mitochondrial Protein Quality Control Pathways in Fibroblasts with the 11778G>A Mutation”. This study extended the traditional genetic approach for studying mitochondrial diseases into the proteome era. The authors compared the proteins produced in 3 different LHON pedigrees to 5 types of unrelated controls using two-dimensional electrophoresis of mitochondrial proteins, followed by mass spec analysis. They identified 17 proteins differentially expressed in LHON versus the controls, and all 17 were down-regulated in LHON lysates of the 11778G>A type. They classified the proteins into two groups: 1) those negatively affecting bioenergetic pathways, and 2) those negatively affecting protein quality control (chaperones). Since LHON has a surprisingly late onset time in patients, we asked Dr. Patcharee whether based on the types of proteins his lab found altered in LHON allowed him to speculate about the relatively late onset of LHON. His research assistant Aung Tun stated “the finding doesn’t allow us to speculate on the late onset of LHON, although there were some differences in the proteins expressed between affected and unaffected cells”. So, although their proteome approach identified protein differences between LHON patients and normal individuals, the types of proteins found to be different did not provide any hypotheses on why LHON shows a relatively late onset.

IVF and Infertility

Emily Caron

With respect to IVF and ART technologies, our review of the literature determined that mitochondrial replacement therapy (MRT) is a recently developed and highly specialized form of IVF. We determined that several steps are similar between MRT and IVF, including egg stimulation, egg retrieval, fertilization in vitro, embryo growth, and embryo implantation. So, we deduced that some of the problems occasionally encountered with IVF technology may also apply to MRT. Our review of the IVF literature determined that some hormone treatments used as a trigger shot to facilitate egg isolation sometimes induce ovarian hyper-stimulation syndrome (OHSS), and that in some cases the percentage of live births is slightly decreased and pre-term births increased, so we focused our questions in these areas.

Some studies indicated that IVF procedures increase the incidence of pre-term births. For example, a 2014 paper was published in *Human Reproduction Update*, 20(3): 439-448, entitled “Neonatal Outcomes Among Singleton Births After Blastocyst versus Cleavage Stage Embryo Transfer: A Systematic Review and Meta-Analysis”. This study performed a systematic review and meta-analysis of pregnancy and neonatal outcomes for singleton IVF births in six observational studies. The authors concluded there was a significantly increased risk of pre-term birth (<37 weeks old) associated with IVF when implanting blastocyst embryos (5-6 days old) into the uterus instead of younger cleavage-stage embryos (1-3 days old). They also concluded that the risk of congenital anomalies may also be higher, but they needed further data to confirm this. To gain a better understanding of how IVF might cause pre-term births, we performed an interview with the study’s corresponding author Dr. ShirDar from the CReATe Fertility Center in Toronto. When asked to explain his lab’s hypothesis for how implanting an “older” blastocyst embryo instead of a younger cleavage-stage embryo might cause pre-term births, and whether he thinks it may allow the embryo to be present in the mother at an earlier stage to receive a broader range of developmental signals, Dr. ShirDar replied, “That is actually a very good question and I’m not sure we have the answer for it. We think that this [increased percent of pre-term births] could be related to the extended time in the culture medium [for the blastocyst embryos] more than the *in vivo* effects of the endometrium (as implantation has not yet occurred). Few studies have focused on the effect of embryo culture”. So, Dr. ShirDar’s lab has not yet proven what aspect of IVF might cause the pre-term births, but says that the longer incubation of the blastocyst embryos in the culture medium might be one explanation. Because MRT also cultures the embryos *in vitro* in a similar medium, this could apply to MRT, so perhaps cleavage-stage embryos should be implanted in MRT procedures, not blastocyst embryos.

Some studies suggest that doctor training and use of improved equipment can increase IVF success rates (the success rates vary depending on the clinic). One example of this is Dr. Marcos Meseguer’s development of a new TMS time-lapse monitoring system for determining the best embryos to transfer to the uterus based on the embryo’s morphology. Dr. Meseguer was a corresponding author on two papers published in 2012 and 2013 in *Fertility and Sterility*. This first paper [98(6): 1481-1489] was entitled “Embryo Incubation and Selection in a Time-Lapse Monitoring System improves Pregnancy Outcomes Compared with a Standard Incubator: A Retrospective Cohort Study”, and the second paper [99(4): 1030-1034] was entitled “Selection of High Potential Embryos Using Time-Lapse Imaging: The Era of Morphokinetic”. These articles

developed a new interesting concept of “morphokinetics” that uses time-lapse imaging to blend a combination of the IVF embryo’s appearance (morphology) with the kinetics of key cellular processes that affect the morphology, for the purpose of improving clinical outcomes. The first study analyzed the reproductive outcome of a new time-lapse monitoring system (TMS) for culturing and selecting IVF embryos. Clinical pregnancy rates were assayed by ultrasound at week-7 for 1,390 TMS embryos versus 5,915 standard incubator embryos. Their data indicated that the new TMS technique significantly improves the clinical pregnancy rate to about 15.7% per embryo transfer. To determine if this new technology would also apply to MRT embryos, we interviewed the study’s corresponding author, Dr. Marcos Meseguer from the Instituto Valenciano de Infertilidad in Spain. After giving him a well-deserved congratulations on his development of the new TMS time-lapse monitoring system, Dr. Meseguer was asked whether he believed this novel approach would work in the future to mitochondrial replacement therapy embryos. Dr. Meseguer replied, “Thank you for your kind words. This new technology will work for all types of IVF cycles, including those with three parent embryos. But keep in mind that mitochondrial replacement therapy is not used routinely in IVF, [so it] still has lots of controversies about its utility... so be critical.” So, in this interview we learned that as we deduced, the new time-lapse monitoring system to closely assay the “morphokinetic” appearance of an IVF embryo to determine its robustness for transfer would indeed apply to MRT embryos. However, MRT is not routinely used yet, so more studies must be done.

Some scientists have studied the serum hormones altered during various supplemental hormone treatments given to the egg providers, and have shown that elevated progesterone levels at the day of the trigger shot results in a decreased pregnancy rates. This topic was addressed in a 2013 paper published in *Human Reproduction Update*, 19(5): 433-457, entitled “Progesterone Elevation and Probability of Pregnancy after IVF: A Systematic Review and Meta-Analysis of over 60,000 Cycles”. The authors analyzed the pregnancy outcomes associated with the levels of serum progesterone elevation (PE) as measure on the day of the final trigger shot. Following the usual ovarian stimulation regime with gonadotrophins (or analogs), the hormone human chorionic gonadotropins (hCG) was administered as the trigger shot to induce final egg maturation. They analyzed serum PE in the mother, receiving either fresh or frozen eggs in more than 60,000 cycles. Their data showed that elevated progesterone levels on the day of the trigger shot correlate with decreased pregnancy rates when using fresh eggs, but not with frozen/thawed eggs. To gain a better understanding of how elevated progesterone levels hinder pregnancy rates, we performed an interview with the study’s corresponding author Dr. Christos A. Venetis from 1st Department of OB/Gyn, Papageourgiou General Hospital in Greece. When asked what their lab’s working hypothesis was on how the elevated progesterone levels at the time of the trigger shot hinders pregnancy, and whether they would speculate on their results would apply to MRT, Dr. Venetis responded that “the proposed theory is that P4 [progesterone-4] affects endometrial receptivity. These results would apply to any case [including MRT] where embryos are transferred to the endometrium and exposed to high P4 levels”. So, here we learned that elevated levels of Progesterone (pregn-4-ene-3,20-dione; abbreviated as P4) in the serum appear to decrease successful IVF pregnancy rates when using fresh eggs but not frozen eggs, and this finding likely will apply to any type of procedure (including MRT) where the embryo is implanted into the uterus. Their lab’s hypothesis is that high levels of serum P4 negatively affect

the lining of the uterus to receive the embryo, although it is still unclear why this only affects fresh eggs not frozen eggs.

Continuing on the theme of supplemental hormone treatments causing side effects, some studies have shown that using a trigger shot of the hormone GnRH α helps eliminate ovarian hyper-stimulation syndrome (OHSS) in IVF patients. One example is a 2011 paper published in *Human Reproductive Update*, 17(4): 510-524, entitled “GnRH agonist for triggering of final oocyte maturation: time for a change of practice”. The authors claim that in fresh IVF cycles, no ovarian hyper-stimulation syndrome (OHSS) was reported after GnRH α triggering (0% risk with GnRH α , with a risk difference of 5%). The delivery rate improved significantly after modified luteal support (6% risk difference in favor of the HCG group). The paper concluded that GnRH α triggering is a valid alternative to hCG triggering, resulting in an elimination of the OHSS syndrome. After correcting for a modified luteal support, the authors showed a non-significant difference of 6% in delivery rate in favor of hCG triggering. So, based on the stronger observation of no OHSS induction, it appears that GnRH α is the better trigger. In order to further our understanding of the possible elimination of OHSS we performed an interview with Professor P. Humaidan from The Fertility Clinic, Sky Regional Hospital, Faculty of Health, Aarhus University in Denmark. Reminding him that some scientists have observed a slight decrease live birth rates (by 6%) using GnRH α as trigger (but no OHSS), we asked Professor Humaidan whether he has observed this decrease in live birth rates in his studies. He replied, “the choice of GnRH α as a trigger shot is now the worldwide protocol of choice for the egg donor, as no OHSS has been reported with this treatment...until now. I just co-authored a now submitted paper from Vietnam, in which we describe a case of OHSS who needed a few days hospitalization. Anyway, this is the first case described out of many thousand GnRH egg donation cycles. This case could be caused by a rare mutation in either the LHR, FSHR, or GnRH α R [receptors]. So yes, at the present, no OHSS cases in the oocyte donor have been reported in the literature [until our new paper comes out]. Regarding your question [about a potential decrease in live birth rates caused by GnRH α], you are right that our 2010 paper showed a decrease (although non-significant) in live birth rate of 7% with GnRH, which superficially favored a hCG trigger, but we then performed a new RCT [randomized control trial], published in 2013, in which we modified the luteal phase support for patients with 14 or fewer follicles to include a second bolus of 1,500 IU hCG on the day of oocyte pick-up. These patients seemed to be the ones who had a higher early pregnancy loss in the 2010 study. In contrast, patients with 15 or more follicles still only had one bolus of 1,500 IU administered on the day of egg pick-up (Humaidan et al., 2010). With this modification for the normal responder (14 follicles or fewer) the ongoing pregnancy rates in both the normal responder (14 or fewer follicles) and the high responder (15 or more follicles) were now non-significantly in favor of GnRH α trigger; however at the cost of two moderate OHSS cases in the normal responder group. So, the handling of the luteal phase after the GnRH α trigger is a matter of fine tuning the dose of hCG given for luteal phase support in relation to the most optimal cut-off value of follicles, and we are there in terms of the reproductive outcome and almost there in terms of OHSS”. When further asked whether he is familiar with MRT, and if so if he believes GnRH α stimulation would work for the MRT patients, he stated, “Yes, I am aware of MRT which obviously demands a mature (MII) oocyte, so from that point of view a GnRH α trigger would also work. And as you are aware, several authors published the retrieval of more MII's [eggs] after a GnRH α trigger compared to a hCG trigger.” Overall, this interview validated our conclusion from the Lit Review that the use of GnRH hormone during IVF procedures as the “trigger shot”

strongly helps eliminate the incidence of ovarian hyper-stimulation syndrome (OHSS) (although his recently submitted paper has identified one rare patient with it). And with respect to whether the use of GnRH as trigger causes a slight decrease in live birth rates, although their 2010 paper showed a potential 7% decrease, this potential decrease became insignificant in their 2013 study when they introduced a second bolus shot in the patient subgroup that previously showed the decrease, and no true effect was observed. In the follow-up question, Dr. Humaidan indicated that as an IVF specialist, he is indeed aware of MRT, and believes that using GnRH as a trigger shot would work well in the MRT cases too, just as with IVF in general.

Our review of the literature identified several studies showing that an increased age of the IVF patient negatively correlates with IVF success rates. One example study was a 2010 paper published in *Human Reproduction Update*, 16(6): 577-89, entitled “Predictive factors in *in vitro* fertilization (IVF): a systematic review and meta-analysis”. This team performed a systematic review of the literature and meta-analysis to identify the most relevant predictors for success in IVF. Nine variables were found that strongly affected IVF success: age, type of patient infertility, indication, duration of the infertility, basal FSH, number of oocytes, fertilization method, number of embryos transferred, and embryo quality. In order to further understand the effect of age on IVF birth success rates, we interviewed Dr. Laura Van Loendershoot from the Center of Reproductive Medicine, Department of Obstetrics and Gynecology, Academic Medical Center, University of Amsterdam in The Netherlands. When asked whether IVF success rates strongly decline with increasing age of the patient and whether she believed MRT will be performed mostly on younger women, Dr. Loendershoot replied, “MRT is not really my field of expertise, but in the case of the three parent mitochondrial gene therapy to prevent transmission of mitochondrial disease, women/couples are not sub-fertile. So the chances of getting pregnant are probably better than with sub-fertile couples. Spontaneous conception depends very much on the female age. Since we don't know whether the mitochondria cause the age-dependent fertility decline, I would say that MRT success is also dependent on the female age.” So, Dr. Loendershoot validated our conclusion from the Lit Review that increased donor age correlates with decreased IVF success rates under normal conditions, and she indicates that MRT patients likely will not have similar underlying fertility problems if undergoing the procedure while relatively young. But she makes a very important point that it's possible that “normal” decreases in fertility can be caused by malfunctioning mitochondria. If this is the case, MRT patients could also show the “subfertility” seen in the older IVF treatments.

MRT Technology *Benjamin Grondin*

This section of the IQP focused on the MRT technology itself, and any problems that might be encountered in the offspring. Our review of the literature quickly focused on concerns with heteroplasmy: the existence of two types of mitochondria in one cell. Some papers showed that low levels of embryo defects might be associated with heteroplasmy, while other scientists argued the very low levels observed following nuclear transfer protocols (<2%) would not be a problem. Other studies indicated that although 1-2% heteroplasmy might result following a nuclear transfer protocol, the heteroplasmy soon becomes undetectable as the cells keep dividing.

To provide more information on this potential problem, we interviewed Dr. Shoukhrat Mitalipov, PhD, of the Division of Reproductive and Developmental Sciences, Oregon National

Primate Research Center, Oregon Health and Science University (Beaverton, OR). Dr. Mitalipov was a corresponding author on a 2009 paper in *Nature*, 461: 367-372, entitled “Mitochondrial Gene Replacement in Primate Offspring and Embryonic Stem Cells”. In this paper, the team established a preclinical (monkey) model for testing mitochondrial replacement protocols in non-human primates. They termed their technique “spindle replacement”. The spindle-chromosomal complex (containing the nuclear genes) was transferred from the genetic monkey mother (in the future a patient with a mitochondrial disorder) to an enucleated donor egg (with normal mitochondria). The reconstructed egg was shown to support normal fertilization, normal embryo development, and apparently healthy offspring. Genetic analyses proved the 3 offspring had nuclear DNA from the “mother” and mitochondrial DNA from the egg donor. No mitochondrial DNA was detected from “mother”, so no heteroplasmy was detected. Dr. Mitalipov was also a corresponding author on a 2013 paper in *Nature*, 493: 627-631, entitled “Towards Germline Gene Therapy of Inherited Mitochondrial Diseases”. In this paper, the authors extended their 2009 spindle transfer (ST) procedure to donated human oocytes. Of 106 human oocytes, 65 underwent ST, and 33 were controls. The fertilization rate was similar in both groups, but the ST group showed elevated numbers of abnormal fertilizations as evidenced by irregular numbers of pro-nuclei. Development to the blastocyst stage, and the isolation rates of embryonic stem cells (ESCs) from the blastocysts were similar between the two groups. All derived ESCs from the ST group showed normal karyotypes and contained exclusively donor mitochondria. Thus, it appears that mitochondria can be efficiently replaced in human eggs, and are capable of normal development to the blastocyst stage. When Dr. Mitalipov was asked whether he was aware of any mitochondrial diseases in monkeys, he replied “Thank you for your note and your interest in our spindle transfer. I am not aware of any nonhuman primate models for mitochondrial disease, but we have produced monkeys carrying donor mtDNA by ST. So, Dr. Mitalipov verified that to his knowledge no one has yet discovered a naturally occurring mitochondrial disease in non-human primates on which to practice the MRT therapy, but his lab has created MRT non-human primates with genetically distinct mitochondria for tracking heteroplasmy.

Continuing to focus on the heteroplasmy problem, we interviewed Dr. Mary Herbert of the Newcastle Fertility Centre at Life, International Centre for Life, Newcastle upon Tyne, NE1 4EP, UK. Dr. Herbert was one of two corresponding authors on a 2010 paper in *Nature*, 465: 82-85, entitled “Pronuclear Transfer in Human Embryos to Prevent Transmission of Mitochondrial DNA Disease”. She showed that mitochondrial DNA (mtDNA) mutations occur in about 1 in 250 live births in the UK, and that about 1 in 10,000 adults have mitochondrial disease. The treatment options are extremely limited, and focus mostly on the symptoms. Using human IVF embryos, the authors show that transfer of the pronuclei between fertilized zygotes results in minimal (<2%) carryover of mitochondrial DNA, and allowed embryo development in vitro at least to the blastocyst stage. Many of the embryos contained no detectable donor mtDNA, so the technique has the potential to treat mitochondrial diseases. When asked whether she thought that a 2% carryover of mitochondria during nuclear transfer would be a problem for the MRT embryo and whether she has ever observed a decline in heteroplasmy over time, she stated “Levels as low as 2% are well below the threshold for mtDNA disease. A recent study of more than 150 pathological point mutations indicated that the probability of disease symptoms (either mild or severe) is very low for mutation loads below 18%. Although other scientists have reported that mtDNA carryover following spindle transfer declines during the proliferation of stem cells, we have not studied this. So, Dr. Herbert indicates that studies have shown that

heteroplasmy levels underneath 18% are not a problem and provide no symptoms, and the 2% observed for MRT nuclear transfers is trivial, and should not be a problem.

This same response (that 2% heteroplasmy will not be a problem) was provided Dr. Douglass M. Turnbull, PhD, of the Mitochondrial Research Group, Institute for Ageing and Health, Newcastle University, NE2 4HH, UK. Dr. Turnbull was a corresponding author on the same 2010 *Nature* paper discussed above and was also a corresponding author on a 2015 paper in the *New England Journal of Medicine*, 372: 885-887, entitled “Mitochondrial Donation: How Many Women Could Benefit?” When asked his opinion on whether a 2% carryover of diseased donor mtDNA would cause any problems due to heteroplasmy in the offspring, and which diseases he thinks are best served by MRT, he replied, “The level of carryover of 2% is of no clinical relevance...many people walk around with this level of heteroplasmy not knowing that they carry it, and it will not cause disease. I think mitochondrial donation [or replacement] could be of benefit to any inherited pathogenic mtDNA mutation”. So, Dr. Turnbull also believes that a mere 2% carryover of diseased mitochondria would be asymptomatic in the MRT offspring, and that many “normal” people have this extent of heteroplasmy without knowing it or being bothered by it. And he says that in his opinion, MRT would be a benefit the offspring of any patient with a mitochondrial disease, he did not see a favorite candidate.

The lab showing that the extent of heteroplasmy declines as the cells continue dividing to the blastocyst stage was Dr. Dieter Egli’s of the New York Stem Cell Foundation Laboratory (New York, NY). Dr. Egli was corresponding author on a 2013 paper in *Nature*, 493: 632-637, entitled “Nuclear Genome Transfer in Human Oocytes Eliminates Mitochondrial DNA Variants”. In this paper, the group demonstrates the feasibility of performing nuclear genome transfer to prevent the transmission of mitochondrial disorders in humans. The technique involved removing the nucleus from the oocyte of a donor female (in the future one with mitochondrial disease, leaving behind her mitochondria) and fusing the nucleus with an enucleated oocyte (with normal mitochondria). The procedure did not appear to reduce developmental efficiency up to the blastocyst stage (at which implantation would proceed). Contaminating mtDNA was initially detected at just below 1%, and then decreased to undetectable levels in blastocysts and embryonic stem cell (ESC) lines derived from the blastocysts. Contaminating mitochondria remained undetectable for more than a year of cell passaging, including manipulations to induce differentiation into a variety of different types of cells. The mitochondria from the procedure had respiratory chain enzyme activities and oxygen consumption rates comparable to mitochondria from the original donor cells. When asked a clarifying point on his procedure and whether the embryos were fertilized for this particular experiment, he replied “No fertilization”. So, Dr. Egli verified that the manipulated egg was not fertilized, it was just stimulated to start dividing so they could follow its development to the blastocyst stage. Their paper shows that although small amounts of mitochondrial heteroplasmy might be present initially in the manipulated egg, the heteroplasmy quickly becomes undetectable as the egg starts dividing.

This same point was verified by Dr. Mark V. Sauer of the Center for Women’s Reproductive Care, College of Physicians and Surgeons, Columbia University (New York, NY). Dr. Sauer was a second corresponding author on the same 2013 *Nature* paper mentioned above for Dr. Egli. When asked to clarify whether the treated oocytes were ever fertilized with sperm (to make a 3-parent embryo) or just chemically treated to follow the ability of the technique to remove the original donor mitochondria, he replied the same as Dr. Egli, “The eggs were not

fertilized; they were artificially activated by parthenogenesis to differentiate”. So, similar to Dr. Egli’s response, Dr. Sauer verified that the manipulated eggs were not fertilized with sperm (which would have made a 3-parent embryo) but instead were artificially activated by parthenogenesis (usually using a chemical like strontium chloride) to allow their divisions to the blastocyst stage. The purpose here was not to create an IVF embryo for implantation, but to create a manipulated egg to study mitochondrial heteroplasmy.

MRT Ethics *Daniel Eckler*

The ethics section of the IQP focused on potential problems with MRT, such as its safety, parental rights (does the donor of the healthy mitochondria retain any parental rights?), its possible use in eugenics (could MRT be used to improve an individual’s genetics other than treating fatal diseases?), and germline modifications (does MRT permanently alter an individual’s DNA?).

Some studies discussed in our review of the literature observed slight increases in birth defects associated with IVF procedures. To help us determine whether MRT offspring may encounter the same types of birth defects, we interviewed Dr. Michele Hansen of the Division of Population Sciences, Telethon Institute for Child Health Research, University of Western Australia (Perth, Australia). Dr. Hansen was corresponding author on a 2013 paper in *Human Reproduction Update*, 19(4): 330-353, entitled “Assisted Reproductive Technology and Birth Defects: A Systematic Review and Meta-Analysis”. This paper represented the first systematic approach in almost 10 years analyzing the literature on birth defects and IVF. The authors analyzed the data from 45 cohort studies for 92,671 assisted reproductive technology (ART) infants versus 3,870,760 naturally conceived infants. Their data showed that ART infants have a higher relative risk (RR) of birth defects (RR 1.32, 95% confidence interval) compared to naturally conceived infants. The risk increased further when restricted to major birth defects (RR 1.42, 95% confidence interval). When asked whether her findings of increased birth defects associated with IVF offspring would apply to MRT offspring, she replied “Thanks for your interest in our paper. I don’t think you can apply the conclusions of that paper to MRT because we examined studies that had reported on birth defects in the children of couples requiring IVF treatment because of their underlying infertility, not because they were trying to avoid the transmission of a particular disease”. This interview makes an important point to be careful trying to apply any negative side effects seen with IVF procedures performed on infertile couples to MRT where the female has a completely different biological problem.

This same topic was asked of Dr. Jennita Reefhuis, an Epidemiologist at the National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS E-86 (Atlanta, GA). Dr. Reefhuis was corresponding author on a 2009 paper in *Human Reproduction*, 24(2): 360-366, entitled “Assisted Reproductive Technology and Major Structural Birth Defects in the United States”. The authors analyzed data from the multi-center National Birth Defects Prevention Study for babies delivered from October 1997 to December 2003. The adjusted odds ratios (AOR’s) associated with each birth defect were calculated at the 95% confidence intervals. For singleton births, ART was associated with: esophageal atresia (adjusted odds ratio 4.5), anorectal atresia (AOR 3.7), cleft lip (with or without a cleft palate) (AOR 2.4), and septal heart defects (AOR 2.1). The cause of the ART

increases in defects was unknown. When asked whether she predicts that the elevated risks seen with ART infants will likely apply in the future to MRT she replied, “Thank you for your question. ART is a rapidly developing field, and in our epidemiological research it takes a few years to collect sufficient data to be able to look into novel medications or treatment methods. Therefore at this time I can really not say whether MRT technology would carry similar risks”. So, Dr. Reefhuis reminds us that because MRT technologies are developing so rapidly, no one knows for sure whether MRT will induce any side-effects.

Not all individuals believe that IVF procedures increase the incidence of birth defects. Dr. Michael J. Davies, MPH, PhD, of the Robinson Institute, Medical School South, Frome Road, Adelaide, SA 5005, Australia, was corresponding author on an interesting 2012 paper in the *New England Journal of Medicine*, 366(19): 1803-1813, entitled “Reproductive Technologies and the Risk of Birth Defects”. The authors analyzed the risks of birth defects appearing before the age of 5 in South Australia. Their results showed an increased odds ratio (OR) for any birth using assisted technology versus normal pregnancies of 1.47 (95% confidence interval). The OR for IVF technology was 1.26, and 1.77 for intra-cytoplasmic sperm injection. Interestingly a patient history of infertility (with or without assisted technology) also significantly increased the overall birth defects, so the authors concluded that their observed increased risks associated with any of the technologies was no longer significant when correcting for patient past history. When asked why he thought that other scientists showed elevated birth defects in their study, Dr. Davies stated “The 2009 Reefhuis study (showing the increases) was a case-control study of a registry, whereas ours was a cohort study of a large population. The effect of this difference in design is that while ours is more representative, it is less able to detect rare events. We did not study individual defect types [such as esophageal atresia] for the main paper, and have not sought to replicate the Reefhuis study for these particular defects. The small numbers can on occasion give rise to relatively large odds ratios with wide confidence intervals, but also with many false negative findings. But nevertheless, we are in broad agreement on cardiovascular and urogenital defects (which includes cases of renal atresia). I have also seen a German study in the last couple of years specifically replicating the anorectal atresia from a specialized registry. I should perhaps add that I have no reason to disbelieve the results”. So, from this interview we learned that when looking for potential birth defects induced by ART procedures, the size of the population studied is very important, and smaller studies can sometimes show potentially large effects that may not pan out in larger population studies, especially when analyzing rare type events. However, Dr. Davies supported the earlier 2009 study, and had no reason to doubt the data shown, and in fact cited a German study who found the same increases in anorectal atresia as the 2009 study

Some studies have examined ART children for side-effects at ages older than most other studies have examined. One such study was done by Dr. Roger Hart of the School of Women’s and Infant’s Health, University of Western Australia (Perth, Australia). Dr. Hart was corresponding author on a 2013 paper in *Human Reproduction Update*, 19(3): 232-243, entitled “The Longer-Term Health Outcomes for Children Born as a Result of IVF Treatments: Part-I: General Health Outcomes”. The motivation for the authors study was the limited amount of data for IVF infants beyond the first year of life. The authors analyzed published studies from January 2000 to April 2012, and found potentially an increase in IVF infant elevated blood pressure, elevated fasting glucose, elevated body fat, advancement of bone age, and increased subclinical thyroid disorder. Interestingly, based on the types of disorders observed, the authors

predict the IVF infants may in the future have increased problems related to cardiovascular disease and metabolic disorders. They recommend further studies be performed long-term. When asked whether his findings might also apply to MRT infants, he stated “Possibly- but there is no evidence to support this”. So, in his short response, Dr. Hart informs us that MRT side-effects could in theory be similar to those he observed in his 2013 longer-term study of IVF babies (including potentially an increase in IVF infant elevated blood pressure, elevated fasting glucose, elevated body fat, advancement of bone age, and increased subclinical thyroid disorder), but so far there is no evidence of this.

Dr. Mert Ozan Bahtiyar has observed congenital heart defects with IVF infants. He is associated with Yale-New Haven Hospital, 20 York Street, West Pavilion, Suite 404 (New Haven, CT). Dr. Bahtiyar was the main author of a 2008 paper published by Yale, and featured on ScienceDaily.com titled “Congenital Heart Defects Increasing Among IVF Twins”. The paper suggests that single babies born of IVF procedures show no increase in congenital heart defects (CHD) when compared to the general population, but twins born through IVF procedures show a three-fold increase. Approximately, 30% of 250 women that were studied had twin pregnancies. When asked about the strength of his data, and whether congenital heart defects might be seen in MRT infants, he stated “recent literature supports our overall increase in CHD with IVF. And with respect to CHD’s and MRT infants, I am familiar with MRT, but being such a new technology am not even sure how routinely it is used in clinical practice, nor am I sure what would be the impact of MRT on CHD frequency.” So, Dr. Bahtiyar verified that the recent scientific literature supports his earlier observations of an increase in congenital heart defects (CHD) for children born of IVF procedures. And with respect to MRT, like most practicing physicians, he has no direct experience with the technique (it is not approved in the U.S.), so he is unsure whether the frequency of CHD would also increase with MRT infants.

IVF and MRT Legalities *Maureen Hester*

The legalities section of the IQP focused on the role of religious views on IVF, the current status of the MRT laws in England, the steps remaining for its approval in the U.S., problems associated with IVF and MRT tourism, and potential problems associated with laws allowing only male embryo transfers.

The review of the literature related to IVF and various religions indicated that different religions hold different beliefs on whether IVF should be allowed. Especially controversial was the use of 3rd party surrogates, as it unites DNA outside the union of marriage. And conflicting views on IVF can even be found between denominations of a single religion. For example, Islam can be divided into two major denominations, Shi’a and Sunni. While IVF is generally accepted and allowed within Islam for a marriage, what is considered acceptable practice is viewed differently by the two denominations. To help us in this area we interviewed Dr. Marcia Inhorn, the William K. Lanman Jr. Professor of Anthropology and International affairs at Yale University (New Haven, Connecticut). She served as Chair of the Council on Middle East Studies in the MacMillan Center for International and Area Studies (2008-20011), and is the author of both the 2006 article “Making Muslim Babies: IVF and Gamete Donation in Sunni vs. Shi’a Islam” published in *Culture, Medicine, and Psychiatry*, and the 2011 paper “Islam, IVF, and Everyday Life in the Middle East” published in *Anthropology of the Middle East*. When

asked how common the practice of mut'ah is (the idea of temporary marriage to allow the use of a surrogate) for IVF, and whether this temporary marriage, while legal, is really accepted within society or has caused a rift between Shi'ites and the more conservative Sunni population, she responded, "Thank you for your interest in our work on Islam and assisted reproduction. I would refer you to our edited volume, *Islam and Assisted Reproductive Technologies: Sunni and Shia Perspectives* (Inhorn and Tremayne, 2012). Both Sunni and Shia Islam are extremely accepting and supportive of the use of IVF. The big difference is that Shia Muslims are allowed to use third-party donors, whereas [the more conservative] Sunni Muslims are not. Some Shia couples do undertake a temporary "mut'a" marriage between the husband and the single egg donor, but this is not mandatory for most Shia couples, and it is my impression that it has become less common over the years. I hope this answers your questions. BOTH Sunni and Shia Islam are VERY supportive of IVF! The big difference is third-parties, which are basically banned in Sunni Islam." So, Dr. Inhorn explains to us that while both Sunni and Shi'a Muslims are in general very supportive of IVF, the major dividing factor is the Shi'a support of third-party donors, while their more conservative Sunni counterparts do not. The increasing approval of third party donors has even lead to a decreasing use of mu'tah within the Shi'a population, as the use of third-party donors without temporary marriage is more accepted.

Fertility tourism is the travel of citizens of countries that ban specific IVF procedures to other countries that allow it to have the procedure done. With the legal approval of MRT in the UK but not in the U.S. we wondered whether a sort of MRT tourism might be born. Dr. Sven Bergmann, who is employed by the Institute for History of Medicine and Medical Ethics (Berlin, Germany), authored a 2011 paper in *BioMedicine Online* entitled "Reproductive agency and projects: Germans searching for egg donation in Spain and the Czech Republic." When asked if he believes that increases in fertility tourism will eventually result in the revision of laws in the recipient countries to more tightly regulate their procedures, or whether he believes that fertility tourism might increase in England with their approval of MRT, Dr. Bergmann responded, "With respect to whether fertility tourism countries might change their tighten their laws, why should countries that serve as "hot spots" for reproductive mobility like Spain or the Czech Republic change their laws? They [via their] private IVF clinics profit from fertility tourism. In Spain it is also framed like "look, we are so modern (regarding access for treating lesbians and single women), so all these people come to us now". In the last years, there was much more pressure on countries like Germany or Austria regarding their more restrictive laws, resulting in a [legal] case against Austria before the European Court of Justice. The state of Austria won in the second instance, so now all member [EU] countries can implement their own style of law. But the case raised a lot of publicity in German speaking countries about why people here have to leave their country due to reproductive reasons. With respect to your second question about whether fertility tourism might apply to MRT in the future, if MRT is mitochondrial replacement therapy is this treatment really important for so many people? As an anthropologist, I am not expert in this issue. Things can change quickly in reproductive services. When I started my research no one talked about oocyte freezing, now with more success cryopreservation of eggs "social freezing" is debated everywhere." So, in this interview Dr. Bergmann raised the point that there is no crucial reason for countries often visited for fertility tourism to tighten their IVF laws because it would not be in their own interest to do so. He mentions a legal case in Austria which resulted in the courts supporting each EU country designing and enforcing their own IVF laws; this case also lead to a significant amount of publicity for German citizens who had to travel to receive IVF treatments, and whether Germany should change its own policies. In terms of

relating fertility tourism to potential MRT tourism, Dr. Bergmann denotes the fact that MRT will be used far less frequently than IVF, and fertility tourism will not be significant in that case.

Our review of the literature indicated that in the U.S., MRT technology is currently under review by the FDA's an Ad Hoc Committee of the Institute of Medicine (IOM), and the findings are not expected until around April of 2016. To validate our conclusion, we contacted Dr. I. Glenn Cohen of the Petrie-Flom Center for Health Law Policy, Biotechnology, and Bioethics at Harvard University (Cambridge, MA). Dr. Cohen is a corresponding author on a recent 2015 paper in *Science*, 348: 178-180, entitled "Transatlantic Lessons in Regulation of Mitochondrial Replacement Therapy". When asked whether he agrees that within the U.S. the report of the Ad Hoc Committee of the Institute of Medicine on MRT safety is likely not expected until April of 2016, and a declaration by that committee of MRT safety is a very important next step, he responded "Yes that's right. I agree! Although Congress has been trying to move its way into the process as well". So, Dr. Cohen agrees with our conclusion that regarding the approval of MRT in the U.S., a declaration of safety by the Institute of Medicine's Ad Hoc Committee is an important next step, and likely won't occur until April of 2016. And it is possible that the U.S. congress will introduce legislation affecting MRT on its own.

Further assessment of this key issue of MRT in the U.S. was provided by Dr. Mark V. Sauer, MD, of the Department of Obstetrics and Gynecology, and Center for Women's Reproductive Care, Columbia University (New York, NY). Dr. Sauer was a corresponding author on a 2015 paper published in *Fertility and Sterility*, 103(2): 344-346, entitled "Controversies Concerning Mitochondrial Replacement Therapy". In the article, the authors state that the US FDA met in February 2014 to discuss MRT, and collected public comments through May 2014, and has suggested that it will continue to ban MRT in the US until more information is obtained. The authors provided arguments on why MRT clinical studies are ethically justified. When asked about the IOM's report and whether that report is a key step in the approval of MRT in the U.S., Dr. Sauer stated "Yes. The IOM does not have regulatory authority, but we would welcome their endorsement. In the meantime, we will continue to pursue our [MRT] research". So, Dr. Sauer agrees that approval of MRT safety by the IOM's Ad Hoc committee is an important next step for MRT approval in the U.S., and indicated that the IOM does not have any regulatory authority. In the end, the FDA has the authority to approve MRT, not the IOM.

Another key issue with MRT is whether the donor of the healthy mitochondria will have any parental rights to the offspring. England determined she would not, leaving the donors of the two nuclei as the legal "parents". This issue was discussed with Dr. Jeffery Kahn who is the Robert Henry Levi and Ryda Hecht Levi Professor of Bioethics and Public Policy and the Deputy Director for Policy and Administration at the Johns Hopkins Berman Institute of Bioethics (Baltimore, Maryland). Dr. Kahn was also the committee chair on the National Academies 2014 meeting "Ethical and Social Policy Considerations of Novel Techniques for Prevention of Maternal Transmission of Mitochondrial DNA Diseases". One key point addressed by the U.K. MRT legislation is that full parental rights are given to the genetic mother and father (who provided the nuclear DNA during MRT), but not to the egg donor (who provided the healthy mitochondria). When asked whether this important item which should be addressed in the United States, Dr. Kahn responded, "I'm not permitted to discuss our committee's deliberations in advance of our report being issued, which will be in early 2016. You should be clear however that the technology won't be the subject of legislation in the

US but rather under the jurisdiction of the FDA with its usual review and approval processes. Sorry I can't be more specific about your question, but stay tuned." Thus, while Dr. Kahn could not specifically discuss the considerations of the National Academies 2014 committee, he does indicate that the U.S. will not need legislation to approve MRT but will proceed with the usual process of seeking FDA approval.

The potential creation of "designer babies" was another worry held by some individuals. But our interviews quickly dispelled this problem, both because mitochondrial DNA does not specify any key individualistic features of a human being (like nuclear DNA does), and because it is relatively easy to have the FDA not approve any "eugenics" like experiments. Dr. Evan Snyder was a committee member at the FDA's February 26, 2014: Cellular, Tissue and Gene Therapies Advisory Committee Meeting. His lab at the Sanford Burnham Preby's Medical Discovery Institute (La Jolla, California) explores the biology of stem cells. When asked if he believed eugenics would really be a problem with MRT, and whether it could subsequently be regulated by U.S. legislation, Dr. Snyder responded, "Thanks for your interest. I agree with you on both accounts. Eugenics is not a problem here [for MRT], and any attempts to extend the techniques beyond minimizing the transmission of devastating diseases can be restricted by federal commissions, internal review boards (IRBs), and/or laws." Thus, Dr. Snyder's response supports the conclusion that the public's fear of eugenics is unfounded, and to assuage any further fears MRT's use for "eugenics" could be blocked by legislation, federal commissions, and review committees, who could restrict MRT to be used exclusively for the prevention of devastating mitochondrial diseases.

Some studies have suggested enacting laws restricting MRT to allow the transfer and implantation of only male embryos, which initially confused us because both males and females suffer from mitochondrial diseases, and both types of embryos carry the same extent of heteroplasmy. Dr. Andy Greenfield has been a Programme Leader at the Medical Research Council (Harwell, United Kingdom) since 1996, and he likely is knowledgeable about England's HFEA proceedings as they approved MRT. When asked if he believes that the implantation of only male embryos would actually decrease the side effects of MRT and which diseases he believed should be a priority for MRT, Dr. Greenfield responded, "The idea that we should create only male offspring after MRT is based on the idea that these [males] would not transmit mtDNA to the next generation. So, any residual heteroplasmy would not be an issue for subsequent generations (as they might be for females). There would otherwise be no predicted differences between males and females. And with respect to [mitochondrial disease] priorities: that really is a clinical decision. As you know, mtDNA mutations can have a wide range of effects, with severity depending on the mutation, the genetic background of the individual, levels of mutant mtDNA and the tissues most affected. These are questions for the HFEA's statutory approvals committee that will ultimately decide on an individual basis whether MRT is justified or not. If a woman has previously had a seriously affected child, then she would be a high priority. But there are also other criteria to consider." So, Dr. Greenfield's response indicates that the reason for allowing only male embryos to be transferred to a uterus following MRT is because their mitochondria, regardless of the extent of heteroplasmy, would not be passed down to their offspring (whether healthy or unhealthy) due to maternal inheritance. The reasoning appears to be independent of males having the same 1-2% of heteroplasmy as females, and has more to do with the fact that fathers do not pass mitochondria to their offspring. He also points out that the priority of diseases will likely be on a patient-to-patient basis, decided by the HFEA's statutory approvals committee, not on a specific disease priority basis.

CONCLUSIONS / RECOMMENDATIONS

Based on the research performed for this project, our team has made several conclusions and recommendations.

Which Mitochondrial Disease?

With respect to which specific mitochondrial disease might best be served by MRT, our interviews with several mitochondrial disease experts concluded that all types of mitochondrial diseases would be treatable by MRT, not just one type, as long as the mutation lies in mtDNA not in nuclear genes encoding mitochondrial proteins (which would remain unaffected by MRT). Although some interviewees pointed out that MRT affects the offspring not the original patient, those interview responses occurred before the late summer 2015 publication of a technique deriving stem cells from MRT patients that might be used in the future for treating the patients themselves (Ma et al., 2015).

Heteroplasmy

One of the most important concerns identified in our MRT research was the worry by some individuals that heteroplasmy would negatively affect MRT offspring. Heteroplasmy is the existence of two different types of mitochondria inside one cell. During MRT, this can potentially occur with the carry-over of small amounts of diseased mitochondria with the nuclear sample during its microinjection into the healthy egg. Interviews with mitochondrial disease experts indicated that heteroplasmy levels below 18% is asymptomatic, as the WT mitochondria are sufficient to drive development and cell function. And some interviewees argued that 1-2% heteroplasmy levels naturally occur in “normal” individuals with no obvious symptoms. The 1-2% levels are also far less than the level of heteroplasmy observed in the cited animal experiments where it was determined to be a problem. And experiments done with human embryos have shown that the levels of heteroplasmy actually decrease further as the cells keep dividing. So, based on our research, we conclude that the mere 1-2% heteroplasmy resulting from MRT procedures should not be a problem and should provide no negative symptoms.

Problems with IVF

MRT is a type of IVF procedure, so we predicted that some issues seen with IVF may also apply to MRT. Based on our research, the IVF findings that likely will also apply to MRT include: 1) a negative correlation of patient’s age with IVF success rates, 2) the use of GnRH hormone as a trigger shot during egg maturation to eliminate ovarian hyper-stimulation syndrome (OHSS), 3) implanting early cleavage-stage embryos instead of blastocysts to increase success, and 4) the use of the new time-lapse video morphokinetic procedure for monitoring

embryos that analyzes key morphological events over time to help identify healthy embryos for transplant.

While some of the IVF findings will likely apply to MRT, not all IVF procedures will. One interviewee whose own research showed an increase in birth defects associated with IVF, cautions that IVF findings may not always pertain to MRT because the two patient populations are very different: IVF couples have underlying fertility problems which affect IVF success outcomes, while MRT patients suffer from a very different type of disease. Another key interviewee pointed out that MRT is a rapidly developing field, and with epidemiological research it takes years to collect a sufficient amount of data, so at this time it is very difficult to say whether MRT technology would carry similar risks. But in any case it will be important to monitor MRT offspring long-term to determine potential effects not only from the beginning but also through middle age.

MRT and Modification of the Germline

With respect to bioethics, the main ethical reason MRT is controversial is it modifies the DNA of the offspring such that it is passed on to future generations. And this modification is done without the individual's (offspring) consent. Previous types of human gene therapy experiments corrected a specific gene for one individual's DNA to treat a specific genetic disease, but in those cases, the risks were assumed only by a consenting individual (usually a patient who had already exhausted all other forms of treatment for their fatal or debilitating disease). And some individuals worry about eugenics, the practice of using gene alterations to improve human genetic features. Based on our research, we conclude that eugenics would not be a problem with MRT. The experts we interviewed agreed with our conclusions that mtDNA makes up only a tiny fraction (0.1%) of the patient's total genome, and importantly has little influence over a human being's *defining* traits. We agree with interviewees who pointed out that once the concerns about safety and efficacy are addressed adequately, it might be considered unethical to deny MRT gene therapies for these diseases that cause severely debilitating and life-threatening conditions in children. In addition, slightly altering a family's mitochondrial gene line already occurs daily naturally during male births when the new nuclear DNA (a combination of the mother and father's nuclear DNAs) is matched with the mother's mitochondria.

IVF Laws

Assisted Reproductive Therapy (ART) is now one of the most highly regulated of all medical practices in the U.S. It is regulated on 3 levels: state, federal, and professional self-regulation. The types of IVF procedures approved vary from country to country, often reflecting local religious influences. With respect to MRT tourism, the travel of patients with mitochondrial disease to countries that have approved the technology, we agree with our interviewees who pointed out that the number of MRT patients will be vastly smaller than general IVF patients, so MRT tourism to England where the technique has been approved likely will not be a problem. With respect to England's MRT debates, we agree that several points discussed there likely will be discussed here in the U.S., including 1) approving MRT only in cases involving mitochondrial diseases, 2) the technology cannot be used for women who are

becoming too old to reproduce and where MRT likely will not work, 3) MRT cannot be used to create "designer babies", and 4) the donor of the healthy mitochondria will not have any parental rights, those rights will be reserved for the two nuclear donors.

Within the U.S., MRT is not approved for human use. Our research indicates that because the technique alters a person's DNA, jurisdiction has been claimed by the U.S. Food and Drug Administration (FDA), especially the FDA's Office of Cellular, Tissue, and Gene Therapies of the Center for Biologics Evaluation and Research. So, unless congress intervenes (and in the summer of 2015 they attempted to do so), the FDA currently has the power to regulate MRT as a form of gene therapy. Under the FDA's regulations, MRT approval will require the performance of phase I, II, and III clinical trials using the standard Investigational New Drug (IND) application method for approval to begin any studies. But to date, the FDA has not officially considered or approved any MRT human clinical trials. Several interviewees agreed with our main conclusion that the FDA is currently awaiting the results of an ongoing investigation of their Ad Hoc Committee of the Institute of Medicine who is considering the safety of MRT, but this report is not due until April of 2016. So, FDA approval is the next step for MRT approval in the U.S. Some scientists believe the Ad Hoc committee will recommend evaluating the long-term health of the MRT monkeys created in Mitalipov's lab, and we agree this is worth considering. Other scientists want to see a standardization of the MRT technique, and this too is worth pursuing.

Overall, we recommend that the FDA approve MRT initially for a small number of patients, and follow their offspring's progress closely for a few years before allowing the procedure to be done on a larger scale. We recommend the FDA approve MRT only for treating mitochondrial disease, and recommend assigning parental rights only to the nuclear donors. In medical research, sometimes human studies are the only way to obtain the final information on safety, and we just have to proceed forward with a few test cases. Animal models are imperfect, and *in vitro* cell studies cannot provide information on long-term side effects, so we just need to move forward.

APPENDIX

Example Questions for Mitochondrial Disease Experts:

1. Our literature search indicates that currently there are no cures for mitochondrial diseases, so doctors can only attempt to alleviate some of the symptoms. Is this still true, and we need a technique like MRT?
2. If MRT were to be approved in the U.S., which mitochondrial diseases do you think should be of highest priority to treat?
3. It is our understanding that mitochondrial diseases can also be caused by mutations in nuclear genes that encode mitochondrial proteins (the nuclear encoded proteins are imported into mitochondria, and function there). Do you agree that MRT likely would not help these women, as the nuclear mutation would remain? Do you agree that genetic testing should be done prior to performing MRT to ensure the problem lies with the mitochondria not the nucleus?

Example Questions for IVF Experts:

1. Side Effects: IVF patients undergoing supplemental hormone treatments (such as GnRH) sometimes develop side-effects like ovarian hyper-stimulation syndrome. Will MRT patients also encounter ovarian hyper-stimulation syndrome? Is this syndrome easily treatable?
2. Patient Selection: Evidence has shown that IVF success rates fall drastically with increased age, so IVF is not for everyone. Will severely diseased women showing strong symptoms of mitochondrial disease be healthy enough for MRT?
3. Prenatal Genetic Diagnosis: PGD is sometimes used during IVF to screen for genetic mutations prior to embryo transfer into the uterus. Will PGD be used with MRT to test whether the implanted embryo contains any remaining diseased mitochondria?
4. Cost: IVF is an expensive procedure, not covered by most U.S. medical plans. Will MRT (involving 3 people, not 2) cost more than IVF? Will medical insurers likely treat MRT (and its potential to help eliminate a fatal disease) differently than IVF (where the couple is looking to have a baby)?
5. Equipment: Some studies suggest that doctor training and the use of specific types of equipment can improve IVF success rates (the success rates vary depending on the clinic). Is this also expected to be the case for MRT?

Example Questions for MRT Experts:

1. What key technical problems do you think were solved to allow the recent MRT successes?
2. Heteroplasmy: Experiments with mice show that engineering them to contain two different mitochondrial genotypes (heteroplasmy) causes reduced physical activity, decreased appetite, a more dramatic stress response, cognitive impairment, higher heart rates, increased body and fat mass indexes, abnormal electrolytes, and abnormal hematological factors. Will heteroplasmy be a problem with human MRT?
 - a. Percent Heteroplasmy: The extent of heteroplasmy likely will be important. The mice mentioned above had as much as 50% heteroplasmy, but most current MRT procedures leave only about 1% mitochondrial carryover (spindle replacement might leave 0% carryover). Will the presence of 1% of diseased mitochondria in the embryo be a problem? Do we need more studies with mice engineered to contain different amounts of heteroplasmy to know the acceptable percent that generates no downstream effects?
 - b. Changes in Heteroplasmy during Development: Some studies performed on human IVF embryos indicate that although heteroplasmy exists immediately following MRT in the cleavage-stage embryos, later the heteroplasmy disappears after more cell divisions. Will this developmental decline apply to all MRT embryos?
 - c. Screening: If heteroplasmy is shown to cause problems, should we pre-screen embryos using prenatal genetic diagnosis (PGD) to determine which embryos lack heteroplasmy, and implant only those embryos?
 - d. Mitochondrial Genotype: Mitochondria differ from each other genetically (haplotypes), but some scientists argue they don't really differ by much, and any differences will not be a problem. Are some genotypes more likely to be problematic matching nuclear genotypes than others?
 - e. Procedures: Some scientists see no evidence of heteroplasmy (for example when performing spindle replacement procedures). Are some techniques less likely to generate heteroplasmy than others?
3. Long-Term Health Problems: Since MRT is a relatively new technique, no study has monitored for potential long-term human health problems into middle age. The earliest mitochondrial injection experiments in humans were done in New Jersey in 1997, so those MRT offspring are now about 18 years old. Of those 17 offspring, one developed autism, two developed Turner Syndrome, one miscarried, and one aborted. Should scientists monitor the long-term health of MRT patients?
4. Male Embryo Transfers: Some scientists have suggested enacting laws to only allow male MRT embryos to be implanted. How would implanting only male embryos decrease the problems of MRT, because male embryos will still have the same levels of heteroplasmy (1%) as female embryos, and males suffer from mitochondrial diseases?

Example Questions for Bioethicists:

1. In 2011, Britain's Human Fertilization and Embryology Authority (HFEA) conducted a scientific/ethical study on MRT, concluding the technique is potentially safe and is ethical. They focused on several problems that likely the U.S. will also need to debate if MRT is to be approved in this country:
 - a. Safety: Is MRT safe? The HFEA review concluded that MRT appears to be safe, but some scientists in the U.S. argue we need more data to prove this, especially for long-term effects. Do you agree?
 - b. Parental Rights: The U.K. decided that only the two nuclear donors will retain parental rights, not healthy mitochondrial donor. Do you agree with this?
 - c. Eugenics: The U.K. law declared that MRT cannot be used to treat anything other than mitochondrial diseases. Some people worry that as we continue to improve MRT it will be used to make designer babies (eugenics) or to treat age-related infertility. But it is our understanding that MRT only replaces mitochondria, and cannot change a person's primary characteristics. mtDNA makes up only a tiny fraction (0.1%) of the patient's total genome and has little influence over a person's *defining* traits. Do you agree?
 - d. Permanent Germline Modification: To some individuals, the strongest objection to MRT is it modifies the DNA of the embryo such that it is passed on to future generations. Previous types of human gene therapy corrected a specific gene for a patient's DNA, and the risk was assumed by that consenting individual (usually a patient who had already exhausted all other forms of treatment for a fatal or debilitating disease). But with MRT, the DNA of the *offspring* would be passed on for *generations*, and the offspring do not provide their consent. Others argue that the parents are acting on behalf of the offspring to treat the mitochondrial disease. What is your opinion on this permanent germline modification?
2. In the U.S., it is our understanding that the Food and Drug Administration (FDA) has formed an Ad Hoc Committee of the Institute of Medicine (IOC) to study the ethical and scientific issues involved in MRT, and their goal is to issue a consensus report by April of 2016. Are you aware of any other MRT ethical studies performed or authorized in the U.S.?

Example Questions for Legal Experts

1. The U.K. Law: Britain's 2015 approval of MRT makes them the first country in the world to approve MRT. Their law bans the use of MRT for any purpose other than treating a mitochondrial disease. It is our understanding that scientifically MRT is only good for this, and could not be used to make designer babies, but do you still think it would be a good idea for the U.S. to enact similar legislation? The U.K. law also gives full parental rights to the nuclear donors, but not to the egg donor who provided the healthy mitochondria. Do you think this point should also be approved in the U.S. legislation?

2. Current Status of the U.S. MRT Debate: It is our understanding that the U.S. is not expecting any formal government report on MRT until about April of 2016, the approximate release time of the FDA's report of the Ad Hoc Committee of the Institute of Medicine (IOM). Do you agree that the next regulatory hurdle for MRT in the U.S. is for it to be declared safe by this FDA-mandated IOM committee?
3. Reproductive Tourism: Now that MRT has been legally approved in the U.K., will their oversight committee (HFEA) allow couples from other countries to travel to the U.K. to undergo MRT?
4. Male Embryo Transfers: Some scientists have suggested enacting laws to only allow male MRT embryos to be implanted. How would this decrease the chances of any side effects from MRT, because male embryos will still have the same levels of heteroplasmy (1%) as female embryos, and males still suffer from mitochondrial diseases?

INTERVIEW PREAMBLE

We are a group of students from the Worcester Polytechnic Institute in Massachusetts, and for our research project we are conducting a series of interviews to investigate problems associated with mitochondrial replacement therapy.

Your participation in this interview is completely voluntary, and you may withdraw at any time. During this interview, we would like to record our conversation for later analysis. We will also be taking notes during the interview on key points. Is this okay with you?

Can we also have your permission to quote any comments or perspectives expressed during the interview? This information will be used for research purposes only, and we will give you an opportunity to review any materials we use prior to the completion of our final report, which will be published on-line in WPI's archive of projects.

If the subject does not agree to be quoted, we will respond as follows: "Since you would not like to be quoted during this interview, we will make sure your responses are anonymous. No names or identifying information will appear in any of the project reports or publications."

Your participation and assistance is greatly appreciated, and we thank you for taking the time to meet with us. If you are interested, we would be happy to provide you with a copy of our results at the conclusion of our study.