FDA BRIEFING DOCUMENT

Cellular, Tissue, and Gene Therapies Advisory Committee

Meeting # 59

February 25-26, 2014

Oocyte Modification in Assisted Reproduction

for the Prevention of Transmission of Mitochondrial Disease

or Treatment of Infertility

***Disclaimer**: This briefing document contains background information prepared by the Food and Drug Administration (FDA) for the members of the Advisory Committee. The briefing document may not present all issues relevant to any specific topic; instead, it is intended to focus on issues identified by the Agency for discussion by the Advisory Committee.*

Table of Contents

I. INTRODUCTION 4

II. MITOCHONDRIAL BIOLOGY 5

A. Mitochondrial function and origin 5

B. Genetic variation within an individual's mtDNA populations 6

C. Mitochondrial inheritance 6

D. Mitochondria in oocytes and early embryos 7

E. Tissue distribution of mitochondria 7

F. Summary of mitochondrial biology 8

III. INHERITED MITOCHONDRIAL DISEASE 8

A. Etiology and pathophysiology 8

B. Natural history 9

C. Diagnosis 9

D. Treatments 10

E. Genetic testing to prevent transmission of mitochondrial disease 10

IV. INFERTILITY 10

A. Prevalence and causes 10

B. Mitochondria and female infertility 11

V. MITOCHONDRIAL MANIPULATION TECHNOLOGIES TO PREVENT TRANSMISSION OF MITOCHONDRIAL DISEASE OR TREAT INFERTILITY 11

VI. SCIENTIFIC INVESTIGATIONS RELEVANT TO THE FEASIBILITY AND PROSPECT OF BENEFIT OF VARIOUS MITOCHONDRIAL MANIPULATION TECHNOLOGIES 12

A. PNT and S/T 12

B. Cytoplasmic transfer/Mitochondrial transfer 13

C. Augmentation of mitochondrial number in oocytes and embryos 13

VII. SAFETY CONSIDERATIONS FOR MITOCHONDRIAL MANIPULATION TECHNOLOGIES FOR BOTH MITOCHONDRIAL DISEASE AND INFERTILITY 15

A. Establishment of criteria to select oocytes and embryos before and after manipulation, and assess the quality of the transferred material 15

B. Potential for inadvertent damage to the manipulated oocyte/embryo 16

C. Carryover of donor mtDNA to the children and heteroplasmy issues 16

D. Nuclear-mitochondrial incompatibility and possible epigenetic modification 18

E. Reagents 19

F. Summary of safety considerations 19

VIII. CLINICAL TRIAL CONSIDERATIONS 19

IX. DRAFT DISCUSSION TOPICS 23

X. GLOSSARY 25

XI. ABBREVIATIONS 28

XII. REFERENCE LIST 29

I. INTRODUCTION

The purpose of this meeting of the Cellular, Tissue, and Gene Therapy Advisory Committee ("the Committee") is to discuss potential future clinical trials of mitochondrial manipulation technologies to prevent transmission of mitochondrial disease from affected women to their children and for the treatment of female infertility.

Many forms of assisted reproductive technology (ART) are currently available and are frequently used by specialists as part of clinical practice. For the purposes of this document, the term "mitochondrial manipulation technologies" refers specifically to those assisted reproductive technology methods under development that are intended to modify the mitochondrial population of an oocyte or embryo. Several specific mitochondrial manipulation technologies have been proposed to prevent the transmission of mitochondrial disease or to treat female infertility. This meeting is intended to discuss issues in the nonclinical and clinical development of the field, and not the development of any specific mitochondrial manipulation technology.

In 2002, a public FDA Advisory Committee meeting was held to discuss ooplasm transfer as a method to treat female infertility. At that time, the Committee identified two significant safety issues that could not be adequately addressed by existing animal and in vitro data: 1) the inadvertent transfer of cellular constituents, such as chromosomes, and 2) the possibility that ooplasm transfer might enhance the survival of certain embryos, increasing the likelihood of children born with significant birth defects [1].

The Committee is being asked to consider new technologies that have become available as well as additional scientific data related to these new technologies. This discussion, which is public and invites public comment, provides transparency for FDA's deliberative processes in approaching the relevant scientific and regulatory issues. FDA recognizes that there are ethical and social policy issues related to genetic modification of eggs and embryos, and that these issues have the potential to affect regulatory decisions; however, such issues are outside the scope of this meeting.

Instead, the Committee is asked to consider scientific, technologic, and clinical issues that would be relevant to future applications in this field. Specific topics for Committee discussion include: 1) the animal and in vitro studies that would be necessary to support the safety and prospect of benefit of mitochondrial manipulation technologies prior to initiation of clinical trials; 2) the risks for the study participants and any children that result from such studies; 3) the design of clinical trials to assess safety and efficacy; and 4) controls and assessments which may be applied to manipulated oocytes and embryos to mitigate risks of mitochondrial manipulation technologies.

To facilitate the Committee's discussion of these specific topics, this briefing document provides pertinent current scientific information on the biology of mitochondria, the unique genetic inheritance of the mitochondrial genome, how mitochondria are distributed among tissues, a brief overview of mitochondrial diseases, the role of mitochondria in oogenesis and early zygotic development, and a discussion of the implications this may have in female infertility. The document also provides an overview of several mitochondrial manipulation technologies and identifies some potential safety concerns for consideration prior to any future clinical trials.

FDA recognizes that the task for this Committee is particularly challenging for several reasons. These include the complexity of the science, the novelty of mitochondrial manipulation technologies, and the absence of a specific regulatory application for consideration at this time. However, the transition from animal and in vitro studies to human clinical trials would be a crucial step in the development of mitochondrial manipulation technologies, and should be made with thorough consideration of the potential risks and benefits for the study subjects and their children. The Committee discussion will inform potential future regulatory deliberations and actions, and thereby assist FDA in its mission to protect those subjects, and the resulting children, of clinical trials of these technologies.

II. MITOCHONDRIAL BIOLOGY

A. Mitochondrial function and origin

Mitochondria are membrane-enclosed cytoplasmic organelles responsible for synthesis of ATP by oxidative phosphorylation (OXPHOS) for use in energy-requiring processes in the cell [2]. Mitochondrial morphology and content differ markedly between tissues, due to developmental and environmental cues and metabolic demand. There are tissue- and organ-specific patterns of fuel use and biosynthetic capability [3], which are manifest in the diversity of protein composition of mitochondria from different sources [4].

Mitochondria have their own DNA, and contain their own transcriptional and protein synthetic machinery. The human mitochondrial genome is small, approximately 16.6 kilobases, and contains 37 genes. However, as each mitochondrion contains 2-10 copies of its circular genome, and each cell contains a large number of mitochondria, an individual cell contains hundreds to thousands of copies of the mitochondrial DNA (mtDNA) genome [5, 6]. Of the 37 genes, thirteen encode polypeptides, 22 encode transfer RNAs, and two encode ribosomal RNAs. All the polypeptides are dedicated to oxidative phosphorylation. Each mitochondrion requires approximately 1500 proteins for its function; most of these are encoded in the nuclear genome and must be imported into the mitochondria. Mitochondrial DNA synthesis is not coupled to nuclear DNA synthesis, and mtDNA has a much higher mutation rate than that of the nuclear genome. The accumulation of mitochondrial mutations throughout an organism's lifespan may contribute to the aging process, cancer, and a number of metabolic diseases [7, 8]. This

accumulation also may contribute to the observed age-related fertility decline in women, which will be further discussed below [9]. In addition, stable pathogenic mutations in mitochondrial DNA are associated with inherited mitochondrial diseases [2, 6] of varying severity. Mitochondrial disease will be discussed in Section III of this document.

B. Genetic variation within an individual's mtDNA populations

When all the sequences of mtDNA within a cell (or tissue, or organism) are identical, this is called homoplasmy. A specific haplotype (genetic variant of normal mtDNA) will accumulate mutations because of the high mutation rate mentioned previously. Given the large number of copies of mtDNA in an individual cell, the probability of sequence heterogeneity among mtDNAs can therefore be substantial. Sequence variants arise and can co-exist in an individual cell with their wild-type counterparts in various proportions, a condition known as heteroplasmy [10]. In contrast, heteroplasmy consisting of different normal haplotypes is not common, due to inheritance patterns described below, and it is not clear what the consequences of such heteroplasmy might be. However, when created experimentally in mouse models, it is associated with abnormalities in pulmonary function, metabolism, and neurological defects [11, 12]. Heteroplasmy of normal and mutant mtDNA is an important factor in the presentation of inherited mitochondrial disease. The severity of a given mitochondrial disease caused by a mtDNA mutation in a specific individual will be influenced by the extent of heteroplasmy: the proportion of normal and mutant mtDNA in that person's cells.

C. Mitochondrial inheritance

In mammals, mitochondria are inherited from the mother. The sperm mitochondria are actively degraded by ubiquitination [13]; thus, the mtDNA genotype (haplotype) is entirely maternal. If the mother is homoplasmic for a single variant of mtDNA, all her oocytes inherit identical mtDNAs, which then comprise the mitochondrial population in her children. However, when multiple sequence variants exist, there is a possibility of unequal partitioning among oocytes, a phenomenon known as the mitochondrial bottleneck [14], where a small number of founder mtDNAs can be over-represented in the pool of mtDNAs of subsequent children [15], both because some mitochondria may be transmitted preferentially and because of the small sample size imposed by the bottleneck. Thus a heteroplasmic mother with low to medium amounts of mutant mtDNA can give birth to children with significantly higher levels of mutant mitochondria[16].

The bottleneck effect is a major contributing factor to our inability to predict the transmission of mitochondrial disease based on the clinical condition of the mother, as her children may inherit mtDNA populations bearing more or less mutant DNA, compared to her mutant load. The bottleneck effect also has ramifications for mitochondrial manipulation technologies that might inadvertently introduce small amounts of different normal haplotypes or mutant mtDNA during

oocyte and embryo modification. This could lead to heteroplasmy, or for pathogenic mitochondrial mutations, these mtDNAs could become overrepresented in the germline of any female child even if they were insufficient to cause mitochondrial disease in the woman herself. Testing the embryo prior to transfer, or the fetus in utero, for mitochondrial disease presents special problems which will be discussed later in this document. Thus, proposals for clinical studies using techniques to modify the mitochondrial population of oocytes or embryos will need to take into consideration the potential for future generations to manifest mitochondrial disease. Female children, but not male children, could transmit a mitochondrial disease to future generations. The Committee may consider whether this difference in the risk to subsequent generations should influence the clinical trial design, e.g., by incorporating gender selection of embryos as a safety measure, by requiring multigenerational follow-up of any female child, or any other precautions.

D. Mitochondria in oocytes and early embryos

The mature human metaphase II oocyte contains hundreds of thousands of mitochondria. Mitochondria in mature oocytes are viewed as structurally undeveloped, with a reduced capacity for respiration [17, 18]. However, the early embryo is dependent on these mitochondria for adenosine triphosphate (ATP) production [19]. Mitochondria are not distributed uniformly throughout the cytoplasm of mature oocytes, but aggregate around the pronucleus and spindle area. They disperse after fertilization [20 21]. Discrete subpopulations of mitochondria, with detectable differences in membrane potential, are also observed subcortically in oocytes and early embryos, suggesting that regional functional differences exist in the mitochondrial pool at these stages [22, 23]. These data speak to the importance of mitochondria in early embryogenesis, and also to their non-random localization in the oocyte and embryo. Mitochondrial manipulation technologies that propose to move the nuclear genome from one oocyte or embryo to another, or augment the resident population of mitochondria in an oocyte, may disturb this organization and adversely affect the oocyte.

E. Tissue distribution of mitochondria

Replication of mtDNA does not resume until after fertilization in humans, most likely around implantation [23, 24]. The mitochondria in the oocyte are therefore partitioned into cells of the blastocyst, and only a few cells of the blastocyst inner cell mass contribute to the embryo itself. This provides an additional opportunity for segregation of mitochondrial variants. In addition, tissue-specific segregation of mtDNA variants is observed postnatally. This segregation is in some cases random, based on mtDNA copy number [25, 26], but allele-specific nonrandom segregation has been observed in humans and in mouse models [27, 28]. This non-random tissue distribution, along with issues related to heteroplasmy and bottleneck phenomena, discussed above, make the presentation of mitochondrial disease caused by mtDNA mutations highly unpredictable, even among individuals carrying the same mtDNA mutations. Some of this non-

random distribution is allele-specific. Therefore, the risks of mitochondrial manipulation technologies may be more predictable for some mtDNA mutations than for other mutations. The Committee might consider whether specific mutations associated with random or non-random tissue distribution are more appropriate for first-in-human (FIH) studies.

F. Summary of mitochondrial biology

The mitochondrial manipulation technologies to be discussed at this meeting propose to change the normal distribution and inheritance of mitochondria. The varied roles of mitochondria, their unusual patterns of inheritance, and their non-random tissue distribution may all need to be considered when evaluating the safety issues posed by any of these methods.

The potential for mitochondrial DNA variants to contribute to a tissue beyond what is predicted by their initial prevalence, particularly in the female germline, is a particular concern, as are the unknown consequences of heteroplasmy in humans. In addition, the oocyte has a specific mitochondrial organization, which may be connected to function, and may be disrupted by manipulation. The effect of such disruption on further development is incompletely understood.

We are asking the Committee to consider this scientific background as it relates to the safety issues outlined in Section VII of this document. As part of the discussion, the Committee might consider to what extent nonclinical studies can adequately assess these issues prior to FIH trials.

III. INHERITED MITOCHONDRIAL DISEASE

A. Etiology and pathophysiology

Inherited mitochondrial diseases comprise a diverse group of disorders that result from mutations, inherited or spontaneous, in either mtDNA or nuclear DNA. Consistent with the focus of this meeting, this section of the document discusses only mitochondrial diseases caused by mutations in mtDNA.

More than 300 pathogenic mtDNA mutations have been identified. These cause a wide range of mitochondrial diseases; all are inherited maternally. Many of these diseases are serious, even life-threatening. Mitochondrial diseases can present at any age, from neonatal to adulthood. The clinical manifestations are heterogeneous, ranging from lesions within a single tissue or anatomical structure, such as the optic nerve in Leber's hereditary optic neuropathy (LHON), to complex multisystem disorders --- for example, involvement of skeletal muscle, heart, and optic nerve in Kearns-Sayre syndrome. For many of these disorders, adult patients commonly present with myopathy, accompanied by diverse symptoms and signs of central nervous system (CNS) involvement, such as ataxia and seizures. Pediatric patients frequently present with delay in psychomotor development, generalized hypotonia, lactic acidosis, and signs of cardiorespiratory failure.

Homoplasmic mtDNA mutations in women are transmitted to all children. The transmission of heteroplasmic mtDNA mutations is more complex due to several factors, such as the mitochondrial bottleneck discussed in the previous section, which can cause a variable amount of mutated mtDNA to be transmitted to each child [29].

Mitochondrial diseases are rare, and the prevalence varies among different diseases. For the majority of mtDNA diseases, the exact prevalence is difficult to estimate with precision because of the clinical heterogeneity and number of disease-causing mutations. The design of clinical trials may be limited by the rarity of these disorders, and particularly by the rarity of any specific mutation. In clinical trials for prevention of transmission of mitochondrial disease, the Committee should consider that eligibility criteria could limit enrollment to women with specific mitochondrial mutations, clinical manifestations, disease severity, extent of heteroplasmy, and other factors. Such eligibility criteria would determine the size of the study population, and thus may influence the feasibility of any proposed clinical trial.

B. Natural history

The natural history of mitochondrial diseases varies with the specific mutation and mutation load [30]. At the present time, there are no established precise correlations between mtDNA genotypes and clinical manifestations. Some of the causes of clinical variability can be explained in part at the molecular level by several factors, which have been discussed in Section II of this document. Therefore, maternal genotype and clinical characteristics do not reliably predict the severity and course of disease in the children. This lack of predictability has implications for selection of subjects for a clinical trial designed to study the safety and efficacy of a given mitochondrial manipulation technology.

C. Diagnosis

For patients who present with typical characteristics of a specific mitochondrial disease, the diagnosis can be made clinically and confirmed by molecular genetic testing of mtDNA extracted from a blood sample. For many other patients, a more detailed, structured approach is needed. Depending on the disease, this approach may include family history, blood and/or cerebrospinal fluid lactate concentration, neuroimaging, cardiac evaluation, muscle biopsy for histologic or histochemical evidence of mitochondrial disease, and molecular genetic testing for a mtDNA mutation.

Some commercial laboratories even provide whole mitochondrial genome analysis. However, even with the evolution of molecular genetic diagnostic testing for mitochondrial disease, diagnostic challenges remain. The ability to accurately identify specific mitochondrial mutations may be critical in the design of clinical trials in mitochondrial disorders. For example, such information may be useful to identify the mitochondrial mutations that would make a woman

eligible for these novel mitochondrial manipulation technologies, to screen potential oocyte donors, and to monitor for mitochondrial mutations in the embryos and children.

D. Treatments

There are no FDA-approved treatments for mitochondrial disease. Current treatment options are largely supportive. As examples, visual aids and occupational rehabilitation are provided to patients with LHON; conventional anticonvulsant therapy to control seizures and physical therapy to help impaired motor function are used in patients with myoclonic epilepsy with ragged red fibers (MERRF). Many experimental interventions have been tested, with limited success. Approaches have included modulation of respiratory chain function (e.g., supplementation with carnitine), removal of noxious metabolites (e.g., lactate), treatment of symptoms (e.g., seizures), and exercise [31] .

E. Genetic testing to prevent transmission of mitochondrial disease

Currently, the only methods to prevent transmission of mitochondrial disease to the children of affected mothers are preimplantation genetic diagnosis (PGD) and prenatal testing in utero to test embryos created by in vitro fertilization. However, these methods are applicable only to some women who have mitochondrial disease due to certain known mtDNA mutations, and in whom the proportion of mutant to normal mtDNA is low (low level of heteroplasmic mtDNA mutation). The discriminatory power of PDG is constrained both by inherent limits in nucleic acid detection levels for heteroplasmic mtDNA mutations, and potential sampling errors due to the variation in mtDNA haplotypes among blastomeres. Therefore, PGD requires sampling of multiple blastomeres. In addition, individuals can tolerate mutant loads differently; therefore, the information acquired by PGD does not permit a clear prognosis. Prenatal diagnosis by chorionic villus sampling or amniocentesis is also used to screen for transmission of mitochondrial disease. However, both methods test a very small proportion of fetal cells, so sampling errors can occur with these methods as well. Repeat testing often is required for confirmation [32, 33].

IV. INFERTILITY

Infertility is defined by the failure to achieve a successful pregnancy after 12 months or more of "appropriate, timed unprotected intercourse or therapeutic donor insemination" [34].

A. Prevalence and causes

Age is a significant factor in fertility: According to the US Center for Disease Control and Prevention (CDC), approximately one-third of couples in which the woman is over 35 years old have fertility problems. Female risk factors or causes for infertility, other than age, include ovarian and hypothalamic-pituitary dysfunction, inadequate tubal patency, physical

characteristics of the uterus, pelvic adhesions, endometriosis, and other intra-pelvic and systemic conditions and diseases.

B. Mitochondria and female infertility

The quality and quantity of mitochondria in the oocyte might contribute to the developmental competence of the embryo, and mitochondrial factors might be linked to infertility and reproductive aging. However, there is no consensus on the extent that female infertility can be attributed to oocyte and embryo mitochondrial insufficiency. Some studies suggest that reproductive mitochondrial insufficiency may be part of a systemic mitochondrial defect [35].

Studies have assessed the amount of mtDNA necessary to undergo normal development. Wai and colleagues found a clear threshold of > 40,000-50,000 mitochondria for normal post-implantation embryonic development [36]. In humans, the amount of mtDNA in mature oocytes has been measured and reported to correlate with fertilizability of human oocytes. However, wide variation is seen, and there is disagreement as to whether the amount of mtDNA is lower solely in women with ovarian insufficiency, or in infertile women in general [37-39].

Studies point to the importance of mitochondria in early mammalian development, but do not agree on the necessary threshold content of mtDNA. Neither is there agreement that mitochondrial function is generally compromised in the reproductive system due to advanced age, rather than as the result of other underlying conditions that impact fertility [23]. The Committee might consider whether there are any types of assessments of mitochondrial number or function could be useful in screening subjects for enrollment into clinical trials in infertility.

V. MITOCHONDRIAL MANIPULATION TECHNOLOGIES TO PREVENT TRANSMISSION OF MITOCHONDRIAL DISEASE OR TREAT INFERTILITY

A brief background on mitochondrial biology and mitochondrial disease has been provided in previous sections of this document. This section, and Sections VI and VII of this document, describe the current state of science for some of the mitochondrial manipulation technologies proposed to prevent transmission of mitochondrial disease or for the treatment of female infertility [5, 15, 40, 41].

As current treatment options for mitochondrial disease are limited, research efforts have focused on preventing transmission of the disease from an affected woman to her children. Various mitochondrial manipulation technologies to augment or replace mutant mtDNA in oocytes or early embryos have been proposed [5, 15, 40, 41]. Since abnormal mitochondria in the oocyte could be related to infertility, these methods might also improve in vitro fertilization (IVF) outcomes for infertile women. Some examples of these mitochondrial manipulation technologies are briefly described below.

Pronuclear transfer and spindle transfer are two methods that have been proposed to potentially completely replace mutant mitochondria. Pronuclear transfer (PNT) is the transfer of the male and female pronuclei from a fertilized oocyte (zygote) into a stage-matched enucleated donor zygote, followed by IVF. Metaphase spindle transfer (S/T) is the transfer of female nuclear genetic material from an oocyte into an enucleated donor oocyte, containing normal mitochondria, followed by IVF. Such methods could produce an embryo containing the nuclear DNA from a woman affected with mitochondrial disease, male nuclear DNA, and normal mtDNA from a donor.

Alternatively, the mitochondrial population of an oocyte could be augmented by cytoplasmic transfer/mitochondrial transfer. Cytoplasmic transfer, also called ooplasm transfer, is conducted by injection of a small amount (5-15%) of cytoplasm from a donor oocyte into a recipient oocyte prior to IVF. The injected material includes proteins, RNAs, small molecules, and mitochondria. This method would require transfer of a large number of mitochondria in order to prevent transmission of mitochondrial disease. Therefore, cytoplasmic transfer may not be practical to prevent transmission of mitochondrial disease [42]. However, augmentation of oocytes with ooplasm or purified mitochondria prior to IVF has been suggested as a treatment for infertility.

VI. SCIENTIFIC INVESTIGATIONS RELEVANT TO THE FEASIBILITY AND PROSPECT OF BENEFIT OF VARIOUS MITOCHONDRIAL MANIPULATION TECHNOLOGIES

Development of specific therapeutic products for a particular clinical indication often involves a stepwise approach, with nonclinical "proof-of-concept" (POC) studies (in vitro and animal) performed prior to the conduct of formal safety studies in animals, followed by clinical trials. The principal goal of POC studies is to generate data supporting the feasibility and potential effectiveness of the product or study procedures in a specific indication. Such POC studies also provide some data regarding potential safety of the product or study procedures.
Some of the scientific investigations described in this section of the document might not have been primarily designed to provide POC data to support the development of the various mitochondrial manipulation technologies to prevent transmission of mitochondrial disease or to treat female infertility. However, these investigations provide data that may be relevant to such development, and therefore may be useful to inform the Committee deliberations.

A. PNT and S/T

The feasibility of PNT [43] and S/T methods [44] was tested in mice using different strains. The data show that both methods are compatible with normal embryo development and resulted in the birth of healthy offspring. Furthermore, PNT in a mouse model of mitochondrial disease prevented transmission of mutant mtDNA and prevented respiratory defects in the offspring [45]. Recently, Tachibana and colleagues transplanted the metaphase II (MII) spindle between

unfertilized oocytes from two genetically distant subspecies of rhesus monkeys, resulting in four live-born rhesus monkeys from three pregnant females [46].

The feasibility of these methods was also tested in human oocytes or embryos in vitro. PNT was carried out in abnormally fertilized human embryos, which were then allowed to develop further. Some embryos reached the blastocyst stage [47]. Two separate groups have carried out S/T with human oocytes in vitro. These manipulated oocytes were fertilized [48] or activated parthenogenetically [49], and some formed normal blastocysts.

These studies provide preliminary evidence that PNT and S/T methods may be feasible. However, these data cannot be seen as traditional POC studies. With the exception of the aforementioned mouse study conducted by Sato et al[45]., the investigations have not used oocytes or embryos containing abnormal mitochondria. Instead, researchers have performed these methods using different animal strains or species, containing different wild-type mtDNA haplogroups, so that the efficiency of replacement of mtDNA could be assessed based on mtDNA sequence differences. Because most of these studies were not done with models of mitochondrial disease or infertility, it is not clear whether these data provide any support for the potential effectiveness of these methods in humans, for either prevention of transmission of mitochondrial diseases or treatment of female infertility.

B. Cytoplasmic transfer/Mitochondrial transfer

Cytoplasmic transfer was introduced into clinical practice in the late 1990's for patients with recurrent implantation failure attributable to poor embryo development, leading to the births of over two dozen children. Among them, there were two instances of Turner's syndrome, a karyotypic abnormality (45X0), and one diagnosis of pervasive developmental disorder, a classification that includes autism. However, the sample size was too small to draw any reliable conclusions concerning the relationship of the cytoplasmic transfer method to the occurrence of disorders in the children [50]. In addition, it is not clear whether the cytoplasmic transfer had any real beneficial effect on the pregnancy outcomes. Since cytoplasmic transfer was conducted in humans prior to more comprehensive animal studies, there has been continued interest in evaluating the effect of purified mitochondria on improving IVF outcomes, with the ultimate goal to treat female infertility.

C. Augmentation of mitochondrial number in oocytes and embryos

Mitochondria have been isolated, purified, and transferred to mouse and human oocytes [51] without damage to their ability to produce ATP. When purified mitochondria from maternally related oocytes were added to pig oocytes, fertilization rates increased. These rates were positively correlated to increased numbers of mitochondria [52]. Addition of mitochondria from autologous granulosa cells improved blastocyst formation rate and the morphology of bovine embryos that had been fertilized by intra-cytoplasmic sperm injection (ICSI) or activated

parthenogenetically [53]. In another experiment, autologous mitochondria isolated from hepatocytes were transferred into mouse zygotes from young or older (>20 weeks of age) mice. In both groups of mice, the addition of mitochondria resulted in greater numbers of normal blastocysts compared to equivalent-aged zygotes that did not receive mitochondria, suggesting that mitochondrial transfer improved embryonic development [54]. However, although there is some evidence that mitochondrial transfer may have a beneficial effect on early embryo development, the effects of mitochondrial transfer on later embryo growth and the birth of healthy offspring have not been investigated.

An autologous source of mitochondria has been used in several of the reported studies in order to alleviate the concern of heteroplasmy; this issue will be discussed more in Section VII of this document. However, since mitochondria display tissue-specific differences in morphology and proteome [4], and autologous somatic cells may have accumulated age-related mtDNA damage, an autologous somatic source may not be appropriate. Use of a stem cell population might address this concern. An oogonial stem cell population was recently identified in mouse and human ovaries, and these cells might provide an autologous source of mitochondria that could be used to modify oocytes to improve IVF outcomes [55-57]. However, there is disagreement as to the existence of these cells [58-61]. In addition, the effect of mitochondrial transfer derived from these oogonial stem cells on embryo growth and live birth in animals has not been reported. Given the uncertainty about the nature of these cells, it is not clear whether they would be an appropriate source of mitochondria for augmentation of an oocyte mitochondrial population.

There is no published consensus on the quantity of mitochondria that would be necessary to improve developmental outcomes. In addition, while the above studies were conducted in various animal species, none of them can recapitulate female infertility, and there is no consensus as to whether female infertility can be attributed to oocyte mitochondrial insufficiency. Therefore, careful consideration should be given to animal models that can provide insight into any potential beneficial effect of mitochondrial transfer on female infertility.

Limited human data on autologous mitochondrial transfer exist. In a clinical study carried out in Taiwan in women with a history of failed in vitro fertilization, poor embryo viability, recurrent implantation failure, prolonged unexplained infertility, and/or advanced age (over 38 years old), mitochondria from autologous cumulus granulosa cells were transferred into oocytes. An abstract reported that pregnancy rates were improved, spontaneous abortion rates were reduced, and the blastocyst morphology was better. Twenty live births were reported from this study, but no follow-up assessment has been published [62] .

VII. SAFETY CONSIDERATIONS FOR MITOCHONDRIAL MANIPULATION TECHNOLOGIES FOR BOTH MITOCHONDRIAL DISEASE AND INFERTILITY

The full spectrum of risks associated with oocyte/embryo manipulation in preventing transmission of mitochondrial diseases or for treatment of female infertility has yet to be identified. Some identified safety concerns are: 1) establishing criteria to qualify source and recipient cells, to evaluate successful nuclear or mitochondrial transfer, and qualify manipulated embryos prior to uterine transfer, which might include genetic tests; 2) the potential for inadvertent damage to the manipulated oocyte or embryo; 3) carryover of mutant mtDNA from the affected oocyte or zygote to the children, or any other cause of heteroplasmy; 4) nuclear-mitochondrial incompatibility and possible epigenetic modification of nuclear DNA; and 5) qualification of the reagents used to carry out these mitochondrial manipulation technologies [33, 47, 63]. Other risks may depend on the specific mitochondrial manipulation technology. This section of the document describes some of the key safety issues that have been identified.

Although this section is presented as a discussion of safety considerations, many of the issues described in this section also relate to the prospect of benefit of these mitochondrial manipulation technologies in preventing the transmission of mitochondrial disease, or in the treatment of infertility.

A. Establishment of criteria to select oocytes and embryos before and after manipulation, and assess the quality of the transferred material

These mitochondrial manipulation technologies will require careful selection of the oocytes and embryos to be manipulated. For PNT and S/T, a first consideration will be the selection criteria for oocytes from which the nuclear material will be removed. The Committee is asked to consider what parameters might identify oocytes and embryos most likely to result in a healthy live birth as an outcome. The same concern applies for the oocytes and embryos that will serve as mitochondrial donors or recipients of nuclear DNA. Data from cytoplast fusion experiments in cows suggest that certain mtDNA haplotypes are more compatible with a given nuclear genome than others [64]. Therefore, consideration should be given as to whether there is an optimal genetic distance (degree of relatedness) between donor and recipient for these methods.

In addition, all these mitochondrial manipulation technologies require multistep procedures in which manipulations are carried out and intermediate states of the manipulated oocyte or embryo are produced during the process. To use these technologies in a clinical trial, consistent criteria for the success of these steps must be established.

Regarding the transfer of mitochondria to improve oocyte quality, the Committee is asked to consider what methods (e.g., tests of mtDNA integrity) will be useful to identify and purify the cell which will be the source of the mitochondria. Mitochondrial copy number is controlled in cells, and elevated mtDNA copy number has been associated with detrimental effects [65]. The

Committee is also asked to consider the methods to determine the optimal number of mitochondria for transfer, and the methods to measure the success of mitochondrial transfer.

Finally, methods such as genetic tests (e.g., PGD) might be useful to select embryos for implantation. The Committee is asked to consider what genetic tests, if any, might be informative and reduce the risks of mitochondrial manipulation technologies.

B. Potential for inadvertent damage to the manipulated oocyte/embryo

The mammalian oocyte and early embryo have a polar architecture susceptible to disruption [66, 67]; this architecture includes the location of the various populations of mitochondria [20, 22, 23]. The published descriptions of PNT and S/T all cite significant numbers of eggs that fail to fertilize and embryos that develop abnormally [47-49]. The number of normal blastocysts acceptable for transfer must therefore be sufficiently high to provide a reasonable expectation of producing viable offspring. Women with mitochondrial disease or infertility may not have sufficient ovarian reserve to provide large numbers of oocytes for manipulation, and comorbidities may preclude repeated ovarian stimulations. In addition, temperature shifts used during the mitochondrial manipulation technology procedures, as well as the reagents used (discussed below), may cause damage to the oocyte. Of particular concern is damage that might not manifest as failure to fertilize or implant, but would lead to unsuccessful pregnancies or safety concerns for the children produced by these mitochondrial manipulation technologies.

C. Carryover of donor mtDNA to the children and heteroplasmy issues

S/T and PNT involve fusion of a recipient oocyte or early embryo to a karyoplast containing the nuclear material surrounded by a small amount of cytoplasm. This cytoplasm may contain mitochondria, resulting in the transmission of mutant mtDNA from an affected woman to the recipient cell. In a mouse model of mitochondrial disease in which nuclei were transplanted from a mtDNA mutant to a non-mutant mouse strain, Sato and colleagues found that the average amount of mtDNA carryover that could be measured was 2% in nuclear-transplanted zygotes, 11% in mice that were 20-30 days old, and 12% over the next 300 days of life [45]. This pattern indicates significant potential for an increase of mutated mtDNA during offspring development. mtDNA carryover was analyzed after PNT in human embryos. Initial carryover levels were 8.1 ±7.6%. However, when a modified PNT technique was used, the average mtDNA carryover was less than 2%, as determined by a last hot cycle PCR RFLP assay [47]. Tachibana and colleagues, carrying out S/T in primate oocytes, reported that carryover of mtDNA was undetectable in the monkey offspring using an assay whose sensitivity to detect mtDNA heteroplasmy levels was >3% [46]. In another study using human oocytes, these researchers employed the more sensitive ARMS-qPCR assay [68] and showed that the mean mtDNA carryover in human S/T embryos was below 1%. In addition, this group reported only small changes, both up and down, in mtDNA carryover in blood and skin samples out to three years in the four monkey S/T offspring [48]. In

another report on S/T in human oocytes, heteroplasmy was detected initially at levels below 1% by both last hot cycle PCR RFLP and ARMS-qPCR assays, decreased in blastocysts and stem cell lines to 0.31 ±0.27%, and was undetectable after passaging for more than one year [49].

The level of carryover of mtDNA was further compared in the somatic tissues and germlines of monkey S/T fetuses [69]. The fetuses from these embryos had low or undetectable donor mtDNA in somatic tissue. However, two oocytes isolated from the ovaries of these fetuses had 16.2 and 14.1% of carryover of mtDNA, suggesting that the donor mtDNA carried over from the S/T procedure had been somehow magnified in the germline.

These studies of S/T or PNT reported amounts of carryover of mtDNA in the embryos and offspring that were below the level of abnormal mtDNA in an embryo that may be accepted for transfer following PGD. However, these data are limited, and the possibility of carryover of even minute amounts of mutated mtDNA to an offspring remains a concern, due to observed tissue-specific inheritance. As discussed previously, increases in the amount of carryover of mtDNA in the female germline, due to bottleneck magnification, may pose a risk for female children derived from these mitochondrial manipulation technologies to transmit mitochondrial disease to their children. In addition, data on the level of carryover of mtDNA in the majority of these studies relied on mtDNA sequence differences between two different wild-type mtDNA haplogroups; the segregation pattern for pathogenic mutated mtDNA could be different [69].

Heteroplasmy has been raised as a concern with donor cytoplasmic transfer/mitochondrial transfer. Several of the children born from cytoplasmic transfer were examined for post-natal persistence of the donor mtDNA genotype, and three cases were reported where amounts of donor mtDNA exceeded amounts expected from the initial transfer, indicating that donor mitochondria had expanded in number relative to the recipient mitochondrial population [50, 70]. Thus, these children had persistent heteroplasmy of two genotypes of wild-type mtDNA. Further follow-up of these children has not been reported [50]. The long-term health consequences of such neutral heteroplasmy are not understood, although recent data from mice suggest an association with abnormalities in pulmonary function, metabolism, and neurological status [11, 12].

In the situation of autologous mitochondrial transfer to improve oocyte quality for infertility, one issue is whether all autologous mtDNA will be genetically identical. Both segregation of mtDNA variants due to bottleneck mechanisms, and age-related accumulation of mutations, may need to be considered as a potential source of heteroplasmy, which could have adverse consequences, even in an autologous setting.

D. Nuclear-mitochondrial incompatibility and possible epigenetic modification

With S/T or PNT, the mtDNA of the recipient oocyte or embryo may be derived from a mitochondrial haplotype different from the nuclear donor. Therefore, the potential for nuclear-mitochondrial incompatibility (or "mismatch") exists with these mitochondrial manipulation technologies. The mitochondrial proteins encoded by the nuclear genome must collaborate efficiently with those encoded by the mtDNA, particularly for OXPHOS function[71]. Evidence is accumulating that mitochondrial genotypes have differential effects which are dependent on the nuclear genotype; these differential effects could impact both evolutionary fitness and lifespan[72, 73]. Diverse nuclear/mitochondrial genome pairings have been created genetically and conditions of "mismatch" have been associated with defects in physical performance and cognition[74, 75]. Tachibana and coworkers [48] found that the overall health of three-year-old monkey S/T offspring, generated by combining nuclear DNA and mtDNA from two genetically distant subpopulations of rhesus monkeys, was comparable to age-matched controls. The authors also confirmed that ATP levels and mitochondrial membrane potential in skin fibroblasts of these four offspring were similar to those of normal controls. These data are encouraging, but very small numbers of progeny have been assessed, and no tests of exercise performance or cognition against age-matched controls were performed.

Mitochondria may have a role in global epigenetic modification of nuclear DNA[76-78]. Studies in mice have shown conflicting outcomes with regard to epigenetic effects and with regard to the growth of offspring. One study by Reik and colleagues[79] reported aberrant methylation patterns in nucleocytoplasmic hybrid mice after female PNT between DBA/2 and C57BL/6 mice. Adult mice derived from these inter-strain transfers had impaired growth and decreased expression of liver-specific proteins, while female PNTs within a strain showed no altered phenotype. This was interpreted as an epigenetic interaction between the transferred pronucleus and the recipient cytoplasm. A related study found that strain-specific methylation of transgenes occurred at fertilization and persisted during fetal life [80]. However, in another study [81], female PNT was performed between DBA/2 and C57BL/6 mice, and no evidence of growth defects or epigenetic abnormalities was observed, although abnormalities were observed when ooplasm was transferred between the two strains. The authors attributed the notably different outcomes to the embryo culture systems and the sources of the inbred mouse lines between their work and that of Reik et al.[79, 81]. Data suggest that even conventional ARTs, such as IVF and ICSI, might impact the epigenetics of early embryogenesis at some low level, resulting in birth defects [82-84].

The consequences of nuclear-mitochondrial incompatibility, or nuclear-cytoplasmic epigenetic effects, as a result of these mitochondrial manipulation technologies are not fully understood. Since many of the outcomes of epigenetic and other gene expression abnormalities do not manifest until much later after birth, the risks to children born with nuclear-mitochondrial incompatibility as a result of these procedures are unknown. These unknowns should be

considered when selecting the appropriate patient population for human studies. Long-term postnatal follow-up may be needed to address this concern.

E. Reagents

PNT and S/T have employed a number of reagents not used in conventional ART [47-49]. In particular, cytochalasin B, a microfilament disruptor, and nocodozole, a microtubule disruptor, are used during removal of nuclei, or to facilitate karyoplast fusion. These reagents can disturb cellular architecture and disrupt chromosome segregation and cell division. In addition, all the current techniques for PNT and S/T also use an extract of inactivated Sendai virus, to promote cell fusion when isolated karyoplasts are reintroduced into an enucleated recipient oocyte or zygote. The genomic RNA of Sendai virus has been completely inactivated in these reagents, as confirmed by in vitro and in vivo assays, making the possibility of viral infection or proliferation remote, but ancillary materials used in the preparation of this reagent may still be a concern [46]. The isolation of oogonial stem cells uses an antibody[55], which also may harbor adventitious agents or ancillary materials of concern. While all these reagents will be extensively diluted by further processing steps prior to the transplantation of the manipulated embryo to a human subject, the potential detrimental effects to the embryo and/or the child, and to the woman who serves as the gestational carrier, as a result of residual levels of these reagents used in these mitochondrial manipulation technologies are not known.

F. Summary of safety considerations

In the context of the available animal models or other experimental systems for assessment of mitochondrial manipulation technologies, the Committee is asked to discuss the specific objectives and endpoints of animal and in vitro studies that would be necessary to support the safety and prospect of benefit of mitochondrial manipulation technologies prior to first-in-human (FIH) clinical trials in prevention of transmission of mitochondrial disease from an affected woman to her children, and in treatment of infertility.

VIII. CLINICAL TRIAL CONSIDERATIONS

The discussion of clinical development of mitochondrial manipulation technologies for prevention of transmission of mitochondrial disease from affected women to their children, and for treatment of female infertility, will focus on the following topics: 1) the potential risks to the women with either mitochondrial disease or infertility, and to children born as a result of use of these techniques; and 2) the design of clinical trials to assess the safety and efficacy of these mitochondrial manipulation technologies for the two clinical indications.

One of FDA's primary objectives during drug development is to assure the safety of the study subjects (21 CFR 312.22(a)). The risks of mitochondrial manipulation technologies to women

with either mitochondrial disease or infertility, and to their children, remain unknown, as no mitochondrial manipulation technology has been studied in human trials. Some of the potential risks have been discussed in Section VII., "Safety Considerations for Mitochondrial Manipulation Technologies." Potential risks to the women could include: 1) failure to become pregnant; 2) failure to deliver a child; 3) risks associated with the specific mitochondrial manipulation technology procedure; and 4) toxicities of the reagents used in mitochondrial manipulation technologies. Potential risks to their children could include: 1) mitochondrial disease (particularly in women with mitochondrial disease), as a result of carryover of abnormal mitochondria and heteroplasmy; 2) disorders due to nuclear-mitochondrial incompatibility; 3) disorders related to aberrant epigenetic modifications; 4) birth defects and other disorders associated with the specific mitochondrial manipulation technology procedure; and 5) toxicities of reagents used in mitochondrial manipulation technologies. There may be additional risks that are difficult to predict because of limitations in current knowledge.

For a woman affected with a mitochondrial disease, the potential benefit of mitochondrial manipulation technologies is the opportunity to have a genetically related child who does not inherit her mtDNA disease. For a woman with infertility, the potential benefit of mitochondrial manipulation technologies is the opportunity to bear a genetically related child.

Available alternatives for women with either mitochondrial disease or infertility include use of other women's oocytes (donor oocytes), donor embryos for reproductive technologies that are currently used in clinical practice, and adoption. For some women who have mitochondrial disease due to known mtDNA mutations, potential alternative approaches to prevent transmission of that disease to their children have been discussed in Section III, "Inherited Mitochondrial Disease." For women with infertility, alternative therapies are fertility medications, intrauterine insemination, and conventional reproductive technologies that are currently used in clinical practice. The success rates for these procedures, particularly in older premenopausal women, are limited.

Clinical trials are essential to ensure successful translation of these mitochondrial manipulation technologies to clinical practice. For trials designed to assess the safety and efficacy of mitochondrial manipulation technologies, it is important to consider the following for each indication:

1) appropriate enrollment criteria for women with mitochondrial diseases and for women with infertility. For example, for mitochondrial diseases, enrollment criteria might include a specific mutation, disease severity, and extent of heteroplasmy in the affected woman.

Mitochondrial manipulation technologies for the prevention of transmission of mitochondrial disease have the risk that any children will have mitochondrial disease. For any female children, that risk may be amplified in subsequent generations. FIH studies could limit these risks by selecting only male embryos for transfer in mitochondrial manipulation technologies to prevent transmission of mitochondrial disease.

For female infertility, because there is no consensus on the extent that infertility can be attributed to oocyte mitochondrial insufficiency, and because of the uncertainty whether a woman's infertility is associated with the quality and quantity of oocyte mitochondria, enrollment criteria might include exclusion of known causes of the infertility (male and female) other than age, as well as failure of available alternative therapies.

2) the need for, and choice of, a control group for each of these indications. For example, for female infertility, inclusion of concurrent controls might be necessary to demonstrate that these mitochondrial manipulation technologies could be safe and effective in treating some women with infertility.

3) safety monitoring for the women and their children. For example, to protect study subjects in these trials, specific protocols might be necessary to monitor maternal, fetal, and child safety.

4) long-term follow-up of the children. For example, to ensure ethical conduct of long-term follow-up, criteria and appropriate language for informed consent and assent will have to be established prior to enrollment. The informed consent documents would have to explain the uncertainties of outcomes of these mitochondrial manipulation technologies, and describe all alternative available therapies as well.

5) measurements of efficacy for each indication. For female infertility, the percentage of reproductive technology cycles currently in clinical practice that result in live births might be considered as one efficacy endpoint. For mitochondrial diseases, clinical, biochemical, and molecular genetic analyses could be used for efficacy endpoints, both to measure clinical benefit and to help understand the molecular and biochemical mechanisms underlying any beneficial clinical activity.

The complex biology, including such issues as heteroplasmy and the reproductive mitochondrial bottleneck, and diverse clinical manifestations of mitochondrial disorders present challenges for the clinical development of mitochondrial manipulation technologies for the prevention of

transmission of mitochondrial disorders from affected women to their children, and for the treatment of infertility. As with any novel intervention, the risks cannot be fully defined prior to clinical experience, but the proposed mitochondrial manipulation technologies have risks that may be carried through multiple generations. This Committee is asked to consider the available data and assess the risks and potential benefits to the mother and children. In addition, the Committee is asked to make recommendations regarding appropriate trial design, including judicious eligibility criteria, safety monitoring, and long-term follow-up, which may serve to mitigate the risks.

IX. DRAFT DISCUSSION TOPICS

Discussion Topic 1

The goal of mitochondrial manipulation technologies is the prevention of transmission of mitochondrial disease from an affected woman to her children or the treatment of infertility. Prior to human clinical investigations, animal and in vitro studies provide the primary data upon which safety assessment is made. In the context of the available animal models or other experimental systems for mitochondrial manipulation technologies, please consider the specific objectives of studies that would be necessary to support the safety and prospect of benefit of mitochondrial manipulation technologies prior to first-in-human clinical trials. Please discuss the ability of available animal models and/or in vitro methods to address the following:

a. The possibility of inadvertent damage to the manipulated oocyte or embryo.

b. The long-term risks associated with the carryover of abnormal mtDNA and heteroplasmy in the children.

c. The potential for abnormal embryo/fetal growth, resulting in children with significant defects.

Discussion Topic 2

Please discuss the potential risks of mitochondrial manipulation technologies to the women with either mitochondrial disease or infertility, and to the resulting children.

Discussion Topic 3

Please discuss the following elements of the design of first-in-human trials to assess the safety and efficacy of mitochondrial manipulation technologies to prevent mitochondrial diseases in children of affected women, and to treat female infertility:

a. Major enrollment criteria. For example, for trials to prevent transmission of mitochondrial diseases, eligibility criteria might limit enrollment to women with specific mtDNA mutations, 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.

For trials to treat infertility, some types of assessments of mitochondrial number or function might be useful in screening women with infertility for enrollment.

b. The controls (comparators), either concurrent or historical, that should be included in trials to provide evidence of safety and efficacy.

c. Procedures to monitor safety and efficacy during fetal development, in the perinatal period, during early childhood, and thereafter. In addition, for trials to prevent mitochondrial diseases, female children, but not male children, could transmit a mitochondrial disease to future generations; safety monitoring could be extended to subsequent generations of female children.

d. Any measures (including, but not limited to, assent or informed consent of the children) that might be necessary for the ethical conduct of long-term follow-up of the children, and subsequent generations of any female children.

e. Measurements of efficacy.

Discussion Topic 4

Adequate manufacturing controls and monitoring of processes are essential to protect the safety of subjects and to minimize the risks for any children that might result from clinical trials using mitochondrial manipulation technologies. Noting that the specific controls might differ for each process, please discuss controls for and/or methods for assessing the following:

a. The source and characteristics of the mitochondria or other subcellular materials. Examples might include tests of mtDNA or spindle integrity, quantitation of mitochondria for transfer, and methods to measure the success of nuclear genome or mitochondrial transfer.

b. The source of the oocytes or other cells.

c. The reagents used in mitochondrial manipulation technologies (e.g., colchicine; Sendai virus extract).

d. The method(s) for qualifying manipulated embryos prior to transfer, including any genetic tests.

X. GLOSSARY

aneuploidy – an abnormal number of chromosomes within a cell; a type of chromosome abnormality

assisted reproductive technology (ART) – human fertility treatments in which both sperm and eggs are handled outside the body. ART includes in vitro fertilization.

autologous – cells or tissues obtained from the same individual

blastocyst – structure formed during vertebrate gestation, prior to uterine implantation. The blastocyst possesses an inner cell mass (ICM), which forms the embryo, and an outer layer of cells, or trophoblast, which forms the placental tissue.

blastomere – a cell produced by cell division of an early embryo

carryover – for the purpose of this document, carryover means for mitochondria to be transferred with the nuclear genetic material in a spindle transfer or pronuclear transfer procedure.

chorionic villus sampling – a form of prenatal testing that samples the chorionic villus tissue of the placenta for genetic abnormalities. Chorionic villus sampling is done at 10-12 weeks gestation.

cumulus granulosa – somatic cells that surround an oocyte in the preovulatory follicle

cytoplast – a small, membrane-bound portion of cellular material. For the purpose of this document, the cytoplast is the vehicle in which mitochondria are moved between cells.

DNA polymerase – the enzyme that synthesizes DNA

electrofusion – a method using small pulses of electricity to fuse two cells or a cell and a cytoplast

endometriosis – a gynecological condition in which cells from the lining of the uterus (endometrium) appear and flourish outside the uterine cavity, most commonly on the membrane which lines the abdominal cavity, the peritoneum.

enucleate – to remove the nucleus from a cell

epigenetic modifications – modifications of DNA that do not involve changes to nucleotide sequence, such as methylation of the DNA bases, or modification to the proteins (chromatin) that cover the DNA. Epigenetic modifications affect the gene expression of the DNA molecule and can be heritable.

germline – the sperm or sperm precursor cells for the male, and the oocytes or oocyte precursor cells for the female. The germline is set aside from the rest of the fetus early in embryonic development.

haplogroup – a group of similar haplotypes that share a common ancestor having the same single nucleotide polymorphisms (SNP) in all haplotypes

heteroplasmy – to have genetically non-identical mtDNA

homoplasmy – to have genetically identical mtDNA

hypotonia – decreased tone of skeletal muscle

ICSI (intracytoplasmic sperm injection) – an ART method, developed to treat male infertility, in which a single sperm is directly injected into an unfertilized egg to fertilize it.

inner cell mass – the portion of a blastocyst (see above) that is destined to become the fetus

IVF (in vitro fertilization) – combining sperm and eggs outside the body to achieve fertilization

karyoplast – a cytoplast (see above) that contains DNA

karyotype – the number and visual appearance of chromosomes

meiosis – the reductive cell division necessary to create haploid cells in preparation for sexual reproduction in eukaryotes

metaphase II – a stage of meiosis II when the chromosomes are arranged on the metaphase plate, a protein structure, and the nuclear membrane has broken down

metaphase plate – the proteinaceous spindle that facilitates chromosome separation during meiosis

oocyte – unfertilized egg

oogonial – coming from the ovary

oogenesis – the process by which oocytes are created in the ovary

ooplasm – cytoplasm of an unfertilized egg or oocyte

oxidative phosphorylation – metabolic pathway within mitochondria wherein nutrients are oxidized to create chemical energy in the form of adenosine triphosphate (ATP)

parthenogenetically – to activate an oocyte without using sperm, using chemicals or electricity. Parthogenetically activated oocytes will not complete embryonic development, but can be used

to study early developmental events. PCR (polymerase chain reaction) – a technique that amplifies small amounts of DNA across several orders of magnitude, used to detect DNA for genetic testing

preimplantation genetic diagnosis (PGD) – testing an early embryo for genetic defects prior to implantation by removing one or more blastomeres and analyzing the DNA within them

pronucleus – the nucleus of a sperm or egg during the process of fertilization, containing half the number of normal human chromosomes

proteome – entire set of proteins expressed by a genome, cell, tissue, or organism at a certain time

qPCR – quantitative polymerase chain reaction – a method based on the polymerase chain reaction to amplify and simultaneously quantify a targeted DNA molecule

ragged red fibers (RRF) – histopathologic finding associated with mitochondrial dysfunction in muscle, caused by accumulation of dysfunctional mitochondria in the subsarcolemmal region of the muscle fiber and appear as "Ragged Red Fibers" when muscle is stained with modified Gömöri trichrome stain

somatic – referring to cells that do not comprise the germline

spindle apparatus – a subcellular structure made of protein that segregates chromosomes between daughter cells during cell division

transgene – a DNA sequence added by transgenic methods such as homologous recombination to the genome of an organism

trophectoderm – also trophoblast, the outer layer of the mammalian blastocyst after differentiation when the outer layer is continuous with the ectoderm of the embryo

ubiquitination – post-translational modification of proteins with a small protein called ubiquitin that targets the modified protein for degradation wild-type – phenotype or genotype of the typical form as it occurs in nature

zygote – initial cell formed when two gamete cells are joined by sexual reproduction

XI. ABBREVIATIONS

ARMS-qPCR – amplification refractory mutation system – quantitative polymerase chain reaction

ART – assisted reproductive technology

ATP – adenosine triphosphate

CNS – central nervous system

DNA – deoxyribonucleic acid

FDA – Food and Drug Administration

FIH – first-in-human

ICSI – intracytoplasmic sperm injection

IVF – in vitro fertilization

MERRF – myoclonic epilepsy with ragged red fibers

MII – metaphase II

mtDNA – mitochondrial DNA

OXPHOS – oxidative phosphorylation

PCR – polymerase chain reaction

PGD – preimplantation genetic diagnosis

PNT – pronuclear transfer

qPCR – quantitative polymerase chain reaction

RFLP – restriction fragment length polymorphism

RNA – ribonucleic acid

RRF – ragged red fiber(s)

S/T – spindle transfer

XII. REFERENCE LIST

Note: References in **Bold** are provided as PDFs with this Briefing Document.

1. FDA. 2002 BRMAC minutes. http://www.fda.gov/OHRMS/DOCKETS/ac/02/minutes/3855m2.htm . 2013.

2. Wallace, D.C. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev Genet.* **39:359-407.**, 359-407 (2005).

3. Vafai, S.B. & Mootha, V.K. Mitochondrial disorders as windows into an ancient organelle. *Nature.* **491**, 374-383 (2012).

4. Pagliarini, D.J. *et al.* A mitochondrial protein compendium elucidates complex I disease biology. *Cell.* **134**, 112-123 (2008).

5. **Chiaratti, M.R., Meirelles, F.V., Wells, D., & Poulton, J. Therapeutic treatments of mtDNA diseases at the earliest stages of human development. *Mitochondrion.* 11, 820-828 (2011).**

6. Schon, E.A., DiMauro, S., & Hirano, M. Human mitochondrial DNA: roles of inherited and somatic mutations. *Nat. Rev Genet.* **13**, 878-890 (2012).

7. Bratic, A. & Larsson, N.G. The role of mitochondria in aging. *J Clin. Invest.* **123**, 951-957 (2013).

8. Wallace, D.C. Bioenergetic origins of complexity and disease. *Cold. Spring Harb. Symp. Quant. Biol.* Dec 22**.**, 1-16 (2011).

9. Bentov, Y., Yavorska, T., Esfandiari, N., Jurisicova, A., & Casper, R.F. The contribution of mitochondrial function to reproductive aging. *J Assist. Reprod. Genet.* **28**, 773-783 (2011).

10. **Chinnery, P.F. & Hudson, G. Mitochondrial genetics. *Br. Med Bull.* 106:135-59. May 22., 135-159 (2013).**

11. Acton, B.M., Lai, I., Shang, X., Jurisicova, A., & Casper, R.F. Neutral mitochondrial heteroplasmy alters physiological function in mice. *Biol. Reprod.* **77**, 569-576 (2007).

12. Sharpley, M.S. *et al.* Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. *Cell.* **151**, 333-343 (2012).

13. Sutovsky, P. *et al.* Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos. *Biol. Reprod.* **63**, 582-590 (2000).

14. Hauswirth, W.W. & Laipis, P.J. Mitochondrial DNA polymorphism in a maternal lineage of Holstein cows. *Proc. Natl. Acad. Sci U S A.* **79**, 4686-4690 (1982).

15. Poulton, J. *et al.* Transmission of Mitochondrial DNA Diseases and Ways to Prevent Them. *Plos Genetics* **6**, (2010).

16. Bolhuis, P.A. *et al.* Rapid shift in genotype of human mitochondrial DNA in a family with Leber's hereditary optic neuropathy. *Biochem. Biophys. Res Commun.* **170**, 994-997 (1990).

17. Dumollard, R., Duchen, M., & Carroll, J. The role of mitochondrial function in the oocyte and embryo. *Curr Top. Dev. Biol.* **77:21-49.**, 21-49 (2007).

18. Motta, P.M., Nottola, S.A., Makabe, S., & Heyn, R. Mitochondrial morphology in human fetal and adult female germ cells. *Hum. Reprod.* **15 Suppl 2:129-47.**, 129-147 (2000).

19. Dumollard, R., Carroll, J., Duchen, M.R., Campbell, K., & Swann, K. Mitochondrial function and redox state in mammalian embryos. *Semin. Cell Dev. Biol.* **20**, 346-353 (2009).

20. Van Blerkom, J., Davis, P., & Alexander, S. Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. *Hum. Reprod.* **15**, 2621-2633 (2000).

21. Yu, Y., Dumollard, R., Rossbach, A., Lai, F.A., & Swann, K. Redistribution of mitochondria leads to bursts of ATP production during spontaneous mouse oocyte maturation. *J Cell Physiol.* **224**, 672-680 (2010).

22. Van Blerkom, J., Davis, P., Mathwig, V., & Alexander, S. Domains of high-polarized and low-polarized mitochondria may occur in mouse and human oocytes and early embryos. *Hum. Reprod.* **17**, 393-406 (2002).

23. Van Blerkom, J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. *Mitochondrion.* **11**, 797-813 (2011).

24. **Shoubridge, E.A. & Wai, T. Mitochondrial DNA and the mammalian oocyte. *Curr Top. Dev. Biol.* 77:87-111., 87-111 (2007).**

25. Chinnery, P.F., Samuels, D.C., Elson, J., & Turnbull, D.M. Accumulation of mitochondrial DNA mutations in ageing, cancer, and mitochondrial disease: is there a common mechanism? *Lancet.* **360**, 1323-1325 (2002).

26. Elson, J.L., Samuels, D.C., Turnbull, D.M., & Chinnery, P.F. Random intracellular drift explains the clonal expansion of mitochondrial DNA mutations with age. *Am. J Hum. Genet.* **68**, 802-806 (2001).

27. Chinnery, P.F. *et al.* Nonrandom tissue distribution of mutant mtDNA. *Am. J Med Genet.* **85**, 498-501 (1999).

28. Jenuth, J.P., Peterson, A.C., & Shoubridge, E.A. Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nat. Genet.* **16**, 93-95 (1997).

29. **Taylor, R.W. & Turnbull, D.M. Mitochondrial DNA mutations in human disease. *Nat. Rev Genet.* 6, 389-402 (2005).**

30. Huang, C.C. *et al.* Clinical phenotype, prognosis and mitochondrial DNA mutation load in mitochondrial encephalomyopathies. *J Biomed. Sci.* **9**, 527-533 (2002).

31. Kerr, D.S. Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past decade. *Mol. Genet. Metab.* **99**, 246-255 (2010).

32. Bredenoord, A.L., Dondorp, W., Pennings, G., De Die-Smulders, C.E., & de, W.G. PGD to reduce reproductive risk: the case of mitochondrial DNA disorders. *Hum. Reprod.* **23**, 2392-2401 (2008).

33. Nuffield Council on Bioethics. Novel techniques for the prevention of mitochondrial DNA disorders. 1http://www.hfea.gov.uk/docs/2011-04-18_Mitochondria_review_-_final_report.PDF . 2013.

34. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil. Steril.* **99**, 63 (2013).

35. Shamsi, M.B. *et al.* Mitochondrial DNA variations in ova and blastocyst: implications in assisted reproduction. *Mitochondrion.* **13**, 96-105 (2013).

36. Wai, T. *et al.* The role of mitochondrial DNA copy number in mammalian fertility. *Biol. Reprod.* **83**, 52-62 (2010).

37. May-Panloup, P. *et al.* Low oocyte mitochondrial DNA content in ovarian insufficiency. *Hum. Reprod.* **20**, 593-597 (2005).

38. Reynier, P. *et al.* Mitochondrial DNA content affects the fertilizability of human oocytes. *Mol. Hum. Reprod.* **7**, 425-429 (2001).

39. Santos, T.A., El, S.S., & St John, J.C. Mitochondrial content reflects oocyte variability and fertilization outcome. *Fertil. Steril.* **85**, 584-591 (2006).

40. **Craven, L. *et al.* Mitochondrial DNA disease: new options for prevention. *Hum. Mol. Genet.* 20, R168-R174 (2011).**

41. Yabuuchi, A. *et al.* Prevention of mitochondrial disease inheritance by assisted reproductive technologies: prospects and challenges. *Biochim. Biophys. Acta.* **1820**, 637-642 (2012).

42. Thorburn, D.R. & Dahl, H.H. Mitochondrial disorders: genetics, counseling, prenatal diagnosis and reproductive options. *Am. J. Med. Genet.* **106**, 102-114 (2001).

43. McGrath, J. & Solter, D. Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. *Science.* **220**, 1300-1302 (1983).

44. Wang, M.K., Chen, D.Y., Liu, J.L., Li, G.P., & Sun, Q.Y. In vitro fertilisation of mouse oocytes reconstructed by transfer of metaphase II chromosomes results in live births. *Zygote.* **9**, 9-14 (2001).

45. Sato, A. *et al.* Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation. *Proc. Natl. Acad. Sci U S A.* **102**, 16765-16770 (2005).

46. **Tachibana, M. *et al.* Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature.* 461, 367-372 (2009).**

47. **Craven, L. *et al.* Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature.* 465, 82-85 (2010).**

48. **Tachibana, M. *et al.* Towards germline gene therapy of inherited mitochondrial diseases. *Nature.* 493, 627-631 (2013).**

49. **Paull, D. *et al.* Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. *Nature.* 493, 632-637 (2013).**

50. Harvey, A.J., Gibson, T.C., Quebedeaux, T.M., & Brenner, C.A. Impact of assisted reproductive technologies: a mitochondrial perspective of cytoplasmic transplantation. *Curr Top. Dev. Biol.* **77:229-49.**, 229-249 (2007).

51. Van Blerkom, J., Sinclair, J., & Davis, P. Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. *Hum. Reprod.* **13**, 2857-2868 (1998).

52. El Shourbagy, S.H., Spikings, E.C., Freitas, M., & St John, J.C. Mitochondria directly influence fertilisation outcome in the pig. *Reproduction.* **131**, 233-245 (2006).

53. Hua, S. *et al.* Effects of granulosa cell mitochondria transfer on the early development of bovine embryos in vitro. *Cloning. Stem. Cells.* **9**, 237-246 (2007).

54. Yi, Y.C., Chen, M.J., Ho, J.Y., Guu, H.F., & Ho, E.S. Mitochondria transfer can enhance the murine embryo development. *J Assist. Reprod. Genet.* **24**, 445-449 (2007).

55. **Woods, D.C. & Tilly, J.L. The next (re)generation of ovarian biology and fertility in women: is current science tomorrow's practice? *Fertil. Steril.* 98, 3-10 (2012).**

56. Woods, D.C. & Tilly, J.L. Isolation, characterization and propagation of mitotically active germ cells from adult mouse and human ovaries. *Nat. Protoc.* **8**, 966-988 (2013).

57. Zou, K. *et al.* Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nat. Cell Biol.* **11**, 631-636 (2009).

58. Ghazal, S. Oogonial stem cells: do they exist and may they have an impact on future fertility treatment? *Curr Opin. Obstet. Gynecol.* **25**, 223-228 (2013).

59. Notarianni, E. Reinterpretation of evidence advanced for neo-oogenesis in mammals, in terms of a finite oocyte reserve. *J Ovarian. Res.* **4**, 1-4 (2011).

60. Oatley, J. & Hunt, P.A. Of mice and (wo)men: purified oogonial stem cells from mouse and human ovaries. *Biol. Reprod.* **86**, 196 (2012).

61. Telfer, E.E. & Albertini, D.F. The quest for human ovarian stem cells. *Nat. Med.* **18**, 353-354 (2012).

62. Tzeng, C.R. *et al.* Mitochondrial transfer (MIT) into oocyte from autologous cumulus granulosa cells (cGCs). *Fertility and Sterility* **82**, S53 (2004).

63. St John, J.C. & Campbell, K.H. The battle to prevent the transmission of mitochondrial DNA disease: is karyoplast transfer the answer? *Gene Ther.* **17**, 147-149 (2010).

64. Bowles, E.J., Tecirlioglu, R.T., French, A.J., Holland, M.K., & St John, J.C. Mitochondrial DNA transmission and transcription after somatic cell fusion to one or more cytoplasts. *Stem Cells.* **26**, 775-782 (2008).

65. Ylikallio, E., Tyynismaa, H., Tsutsui, H., Ide, T., & Suomalainen, A. High mitochondrial DNA copy number has detrimental effects in mice. *Hum. Mol. Genet.* **19**, 2695-2705 (2010).

66. Antczak, M. & Van, B.J. Temporal and spatial aspects of fragmentation in early human embryos: possible effects on developmental competence and association with the differential elimination of regulatory proteins from polarized domains. *Hum. Reprod.* **14**, 429-447 (1999).

67. Antczak, M. & Van, B.J. Oocyte influences on early development: the regulatory proteins leptin and STAT3 are polarized in mouse and human oocytes and differentially distributed within the cells of the preimplantation stage embryo. *Mol Hum. Reprod.* **3**, 1067-1086 (1997).

68. Newton, C.R. *et al.* Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic. Acids Res.* **17**, 2503-2516 (1989).

69. Lee, H.S. *et al.* Rapid mitochondrial DNA segregation in primate preimplantation embryos precedes somatic and germline bottleneck. *Cell Rep.* **1**, 506-515 (2012).

70. Brenner, C.A., Kubisch, H.M., & Pierce, K.E. Role of the mitochondrial genome in assisted reproductive technologies and embryonic stem cell-based therapeutic cloning. *Reprod. Fertil. Dev.* **16**, 743-751 (2004).

71. Woodson, J.D. & Chory, J. Coordination of gene expression between organellar and nuclear genomes. *Nat Rev Genet.* **9**, 383-395 (2008).

72. Clancy, D.J. Variation in mitochondrial genotype has substantial lifespan effects which may be modulated by nuclear background. *Aging Cell.* **7**, 795-804 (2008).

73. Lane, N. Mitonuclear match: optimizing fitness and fertility over generations drives ageing within generations. *Bioessays.* **33**, 860-869 (2011).

74. Nagao, Y. *et al.* Decreased physical performance of congenic mice with mismatch between the nuclear and the mitochondrial genome. *Genes. Genet Syst.* **73**, 21-27 (1998).

75. Roubertoux, P.L. *et al.* Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. *Nat Genet.* **35**, 65-69 (2003).

76. Bellizzi, D., D'Aquila, P., Giordano, M., Montesanto, A., & Passarino, G. Global DNA methylation levels are modulated by mitochondrial DNA variants. *Epigenomics.* **4**, 17-27 (2012).

77. Wallace, D.C. & Fan, W. Energetics, epigenetics, mitochondrial genetics. *Mitochondrion.* **10**, 12-31 (2010).

78. Smiraglia, D.J., Kulawiec, M., Bistulfi, G.L., Gupta, S.G., & Singh, K.K. A novel role for mitochondria in regulating epigenetic modification in the nucleus. *Cancer Biol. Ther.* **7**, 1182-1190 (2008).

79. Reik, W. *et al.* Adult phenotype in the mouse can be affected by epigenetic events in the early embryo. *Development.* **119**, 933-942 (1993).

80. Pickard, B. *et al.* Epigenetic targeting in the mouse zygote marks DNA for later methylation: a mechanism for maternal effects in development. *Mech. Dev.* **103**, 35-47 (2001).

81. Cheng, Y. *et al.* Effects of ooplasm manipulation on DNA methylation and growth of progeny in mice. *Biol. Reprod.* **80**, 464-472 (2009).

82. DeBaun, M.R., Niemitz, E.L., & Feinberg, A.P. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum. Genet.* **72**, 156-160 (2003).

83. Gosden, R., Trasler, J., Lucifero, D., & Faddy, M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. *Lancet.* **361**, 1975-1977 (2003).

84. Wrenzycki, C. *et al.* Epigenetic reprogramming throughout preimplantation development and consequences for assisted reproductive technologies. *Birth Defects Res C. Embryo. Today.* **75**, 1-9 (2005).