

BRMAC Briefing Document for Day 1, May 9, 2002

Ooplasm transfer as method to treat female infertility

Introduction

Several fertility clinics in the United States are experimenting with ooplasm transfer. This technique is hypothesized to enhance fertility in women who have failed conventional *in vitro* fertilization due to poor embryo development. It involves injection of cellular material (cytoplasm or ooplasm) from a donor egg into the mother's unfertilized egg prior to fertilization *in vitro*. The transferred material includes proteins, RNAs, small molecules, and organelles. Prominent among these are mitochondria, the organelles responsible for cellular aerobic respiration. Mitochondria contain their own genetic material, as they are thought to have evolved from primitive bacteria. Thus, when ooplasm is transferred, mitochondrial DNA from the donor is transferred to the recipient egg. Several recent scientific reports document that some children born following use of this technique do, indeed, carry genetic material from three separate individuals: their biological parents and the ooplasm donor. The technique is therefore *de facto* germ line gene transfer and represents the first reported case of "human germline genetic modification" [1]. This crosses a line drawn by many scientists and bioethicists at altering the genetic profile of unborn children [2;3] and, as a result, has occasioned attention in scientific publications and the popular press.

Ooplasm transfer in the United States

Over two dozen births attributed to ooplasm transfer have been reported by three clinics since 1998. As of June, 2001, at least 8 U.S. clinics were offering this procedure. Fresh oocytes, frozen oocytes, and tripronucleate zygotes have all been used as sources of donor cytoplasm [4-6]. Both electrofusion of cytoplasts and direct injection have been investigated as methods of ooplasm transfer. It is not clear what defect is being corrected by this technique. The active component or components of the transferred ooplasm have not been identified, nor is there consensus on this point. There are differences in energy content (ATP) in human oocytes [7] and data that suggest that mitochondrial activity is negatively correlated with advancing age [8]. These data support the concept that mitochondria may confer at least a portion of the proposed fertility-enhancing effect of ooplasm. In rodents, epigenetic modifications of paternal or maternal genomes have been attributed to differences in cytoplasm, indicating that additional activities of ooplasm may also be relevant [9].

Of 30 fertilizations achieved after ooplasm transfer from fresh oocytes, 13 pregnancies were reported [10]. Two fetuses were karyotypically 45, XO (Turner's syndrome). One of these fetuses aborted spontaneously and the other pregnancy was terminated. It is unknown whether a connection exists between these reported aneuploidies and the ooplasm transfer procedure.

Mitochondria and Heteroplasmy

The mitochondrial genome is small, approximately 15-17 kb. It encodes 13 proteins, and contains other non-protein coding genes [11]. Most of the proteins used by the mitochondria are encoded by the nuclear (host) genome and many of these must work in conjunction with proteins produced by the mitochondrial genome. Regulation between the two compartments must therefore be closely coordinated, but the details of this process are still unclear [12].

Mitochondria play roles in programmed cell death (apoptosis) and in ion fluxes, particularly calcium flux. However, the primary role of the mitochondrion is aerobic respiration by oxidative phosphorylation. Thus the mitochondrion is an environment rich in mutagenic oxygen radicals, and mitochondrial DNA (mtDNA) has a mutation rate 5-10X that of the nuclear genome. Mutations in human mtDNA continue to be identified. Many of these are associated with serious disorders, including premature aging, myopathies, neurodegenerative diseases, and diabetes[13].

In mammals, the inheritance of mtDNA is strictly maternal. In humans, mitochondria derived from the spermatozoa are destroyed early in embryogenesis. Therefore, all the mitochondria in the developing embryo derive from the cytoplasm of the oocyte. The embryo normally inherits a single sequence variant of mtDNA, a condition called homoplasmy. Evolutionary biologists speculate that homoplasmy has arisen to avoid cytoplasmic competition between mitochondrial genomes, and to avoid difficulties in coordination between the nuclear and mitochondrial genomes [14;15].

The high mutation rate observed for mtDNA and the apparent lack of variation in a given individual's mitochondrial genotype indicate that a restriction event must occur to eliminate variation in a given individual's mitochondrial genotype. This is referred to as the 'bottleneck' [16;17], and appears to occur during oogenesis. Whether it occurs by selection or stochastically is unresolved [18], but evidence suggests that when organisms are found to contain more than one mitochondrial genotype, a condition known as heteroplasmy, this variation disappears within in a few generations in the individual's offspring, and one genotype prevails.

Homoplasmy therefore is the normal condition in mammals. There have been observations of mitochondrial heteroplasmy at single loci in humans [19]. Humans show mutations in a subset of their mitochondrial populations during aging or in acquired mitochondrial disease, but a marked lack of sequence variation in mitochondrial genotype is most commonly observed in young, healthy individuals.

Segregation patterns of mitochondrial genotypes in artificially created heteroplasmic animals have been analysed. Interestingly, segregation of mitochondrial genotypes was not random. Instead, selection for genetically distinct mitochondrial populations was tissue specific [20]. This type of selection pressure does not seem to be based solely on differences in respiration or in efficiency of replication [21]. Such examples of nonrandom tissue distribution of mtDNA genotypes have also been seen in humans with mutant genotypes leading to disease states. For example, a mutant mtDNA genotype associated with the condition 'mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes' (MELAS) has been found to have a nonrandom tissue distribution [22].

Localization of mitochondria in oocytes and embryos

Oocytes and embryos have polarized cytoarchitecture. A prominent example of such polarization is that mitochondria are not randomly located in oocytes and early embryos. In several mammalian species, including humans, relocation of mitochondria during oocyte maturation has been reported [8;23;24]. Immediately after fertilization, mitochondria are observed to move to a perinuclear position in rhesus monkey and human embryos [8;24;25]. This relocalization is thought to be necessary to support the increased energy requirements of the nucleus, particularly the spindle apparatus.

The partitioning of mitochondria among blastomeres of the early cleaving embryo also is not random [23]. Mitochondrial genotype segregation among blastomeres was observed in artificially produced heteroplasmic mouse embryos [26] and asymmetric distribution of mitochondria has been reported for human embryos, with some blastomeres receiving a greater proportion of the pool of mitochondria than others. Poor developmental potential is observed in blastomeres with low mitochondrial number [23]. In addition, mitochondria of oocytes and early embryos do not appear to be an entirely homogeneous population, as measured by their membrane potential, and may have specific subcellular localizations that reflect differences in function [27]. It should be noted that no mitochondrial replication occurs from fertilization until perhaps as late as implantation, so the early embryo has a limited supply of mitochondria [16].

All these observations support a hypothesis that cells, and particularly oocytes and embryos, adjust mitochondrial density to support specific metabolic demands, and that failure to maintain appropriate mitochondrial distribution can have negative effects on embryonic development.

Mitochondria and ooplasm transfer

In experimental procedures carried out in human embryos, the fate of mitochondria transferred from fresh donor oocytes was monitored by confocal microscopy using fluorescent dyes specific for active mitochondria [1]. Active donor mitochondria were found in the recipient oocyte and segregated to many of the blastomeres of the early embryo. Active donor mitochondria were not evenly segregated throughout the blastomeres after the ooplasm transfer procedure. The proportion of the genotypes of mitochondria from donor and recipient was determined by PCR, using primers from the hypervariable region of the mitochondrial genome. This is the region used by population geneticists and forensic biologists to track mitochondrial genotypes between individuals. Mitochondrial genotypes were monitored in nonviable embryos, amniocytes, placental tissue and fetal cord blood of pregnancies resulting from ooplasm transfer. Donor mitochondria could be detected in multiple numbers of these sites in more than one subject and the tissue distribution of the mtDNA heteroplasmy was variable [28]. Further follow-up indicated that this heteroplasmy persisted in two children at 9 months and 14 months of age, as determined by direct sequencing of DNA from blood samples.

FDA's actions and concerns

In June, 2001 FDA sent a letter to fertility clinics and practitioners in the U.S. identified either by publications on ooplasm transfer or advertisements offering the procedure. We advised practitioners that FDA has jurisdiction over the use of human

cells that have received transferred genetic material by means other than the union of gamete nuclei. We indicated that any further ooplasm transfer protocols should be done under Investigational New Drug (IND) exemptions and that an IND submission to the agency would be required to treat additional patients.

Ooplasm transfer is an experimental technique that has not been evaluated extensively in animals. There has been little oversight of this clinical intervention beyond that of local Institutional Review Boards. There is a paucity of published preclinical data supporting either the safety or the effectiveness of this procedure. As outlined above, the regulation of mitochondria is complex and incompletely understood. However, it is clear that stringent mechanisms have evolved to insure homogeneity of mitochondrial genotypes at the initiation of human development. FDA has concerns about the safety of perturbing this process. In addition, this technique changes the genetic makeup of the resulting offspring. Appropriate follow-up of children born after ooplasm transfer and their progeny must therefore be considered carefully. FDA feels further public discussion is necessary to: 1) evaluate the potential risks of this procedure, 2) recommend how safety should be monitored, 3) assess how efficacy might best be determined, and 4) determine what further nonclinical data will be needed to support additional human clinical trials.

References

1. **Barritt,J.A., Brenner,C.A., Malter,H.E., and Cohen,J.,** Mitochondria in human offspring derived from ooplasmic transplantation. *Human Reproduction* 2001. **16**: 513-516.
2. **Recombinant DNA Advisory Committee.** Prenatal Gene Transfer: Scientific, Medical and Ethical Issues. 2000.
3. **Frankel, M. S. and Chapman A.R.** Human Inheritable Genetic Modifications. 2000. American Association for the Advancement of Science.
4. **Cohen,J., Scott,R., Alikani,M., Schimmel,T., Munne,S., Levron,J., Wu,L., Brenner,C., Warner,C., and Willadsen,S.,** Ooplasmic transfer in mature human oocytes. *Molecular Human Reproduction* 1998. **4**: 269-280.
5. **Lanzendorf,S.E., Mayer,J.F., Toner,J., Oehninger,S., Saffan,D.S., and Muasher,S.,** Pregnancy following transfer of ooplasm from cryopreserved- thawed donor oocytes into recipient oocytes. *Fertility and Sterility* 1999. **71**: 575-577.
6. **Huang,C.C., Cheng,T.C., Chang,H.H., Chang,C.C., Chen,C.I., Liu,J.E., and Lee,M.S.,** Birth after the injection of sperm and the cytoplasm of tripronucleate zygotes into metaphase II oocytes in patients with repeated implantation failure after assisted fertilization procedures. *Fertility and Sterility* 1999. **72**: 702-706.
7. **Van Blerkom J, Davis PW, and , Lee J.** ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo trans. *Human Reproduction* **10**, 415-424. 1995.
8. **Wilding,M., Dale,B., Marino,M., di Matteo,L., Alviggi,C., Pisaturo,M.L., Lombardi,L., and De Placido,G.,** Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. *Human Reproduction* **2001. 16**: 909-917.
9. **Hawes SM, Sapienza C, and Latham KE.** Ooplasmic donation in humans: The potential for epigenic modifications. *Human Reproduction* **14**, 850-852. 2002.
10. **Barritt,J.A., Willadsen,S., Brenner,C., and Cohen,J.,** Epigenetic and experimental modifications in early mammalian development: Part II - Cytoplasmic transfer in assisted reproduction. *Human Reproduction Update* 2001. **7**: 428-435.
11. **Lightowers,R.N., Chinnery,P.F., Turnbull,D.M., and Howell,N.,** Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. *Trends in Genetics* 1997. **13**: 450-455.
12. **Poyton,R.O. and McEwen,J.E.,** Crosstalk between nuclear and mitochondrial genomes. *Annual Review of Biochemistry* 1996. **65**: 563-607.
13. **Wallace,D.C.,** Mitochondrial diseases in man and mouse. *Science* 1999. **283**: 1482-1488.
14. **Cummins,J.,** Mitochondrial DNA in mammalian reproduction. *Reviews of Reproduction* 1998. **3**: 172-182.
15. **Cummins,J.M.,** Mitochondria: potential roles in embryogenesis and nucleocytoplasmic transfer. *Human Reproduction Update* 2001. **7**: 217-228.

16. **Jansen,R.P.S. and de Boer,K.**, The bottleneck: mitochondrial imperatives in oogenesis and ovarian follicular fate. *Molecular and Cellular Endocrinology* 1998. **145**: 81-88.
17. **Chinnery,P.F., Thorburn,D.R., Samuels,D.C., White,S.L., Dahl,H.H.M., Turnbull,D.M., Lightowlers,R.N., and Howell,N.**, The inheritance of mitochondrial DNA heteroplasmy: random drift, selection or both? *Trends in Genetics* 2000. **16**: 500-505.
18. **Jenuth,J.P., Peterson,A.C., Fu,K., and Shoubridge,E.A.**, Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nature Genetics* 1996. **14**: 146-151.
19. **Wilson,M.R., Polansky,D., Replogle,J., DiZinno,J.A., and Budowle,B.**, A family exhibiting heteroplasmy in the human mitochondrial DNA control region reveals both somatic mosaicism and pronounced segregation of mitotypes. *Human Genetics* 1997. **100**: 167-171.
20. **Jenuth,J.P., Peterson,A.C., and Shoubridge,E.A.**, Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nature Genetics* 1997. **16**: 93-95.
21. **Battersby,B.J. and Shoubridge,E.A.**, Selection of a mtDNA sequence variant in hepatocytes of heteroplasmic mice is not due to differences in respiratory chain function or efficiency of replication. *Human Molecular Genetics* 2001. **10**: 2469-2479.
22. **Chinnery,P.F., Zwijnenburg,P.J.G., Walker,M., Howell,N., Taylor,R.W., Lightowlers,R.N., Bindoff,L., and Turnbull,D.M.**, Nonrandom tissue distribution of mutant mtDNA. *American Journal of Medical Genetics* 1999. **85**: 498-501.
23. **Van Blerkom,J., Davis,P., and Alexander,S.**, Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. *Human Reproduction* 2000. **15**: 2621-2633.
24. **Bavister BD and Squirrell JM** Mitochondrial distribution and function in oocytes and early embryos. *Human Reproduction*. **15** Suppl 2, 189-198. 2000.
25. **Van Blerkom,J., Sinclair,J., and Davis,P.**, Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. *Human Reproduction* 1998. **13**: 2857-2868.
26. **Meirelles,F.V. and Smith,L.C.**, Mitochondrial genotype segregation during preimplantation development in mouse heteroplasmic embryos. *Genetics* 1998. **148**: 877-883.
27. **Van Blerkom,J., Davis,P., Mathwig,V., and Alexander,S.**, Domains of high-polarized and low-polarized mitochondria may occur in mouse and human oocytes and early embryos. *Human Reproduction* 2002. **17**: 393-406.
28. **Brenner,C.A., Barritt,J.A., Willadsen,S., and Cohen,J.**, Mitochondrial DNA heteroplasmy after human ooplasmic transplantation. *Fertility and Sterility* 2000. **74**: 573-578.

