Nuclear Donation—Embryonic Health and Wellness Before Conception (Research in Planning)

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Perry,[1] in a recent issue of the New England Journal of Medicine, summarized the progress on human somatic cell nuclear transfer. He cited pioneering research by Hwang et al,[2,3] who transferred somatic-cell nuclei from 8 male and 3 female donors into oocytes whose nuclear genomes have been removed (empty eggs).

Cells containing nuclei from 9 donors developed to the blastocyst stage, whereas cells containing nuclei from the other 2 donors failed. Blastocysts from each of the 9 patients yielded 1 or 2 embryonic stem cell lines for a total of 11 embryonic stem cell lines from 31 blastocysts.

In Hwang et al's experiments[2,3] all the donors were suffering from conditions which are amenable to stem cell therapy: juvenile diabetes, spinal cord injury, etc. Thus, the main goal of somatic cell transfer is to derive pure populations of relevant cells from them in vitro. It is now possible to induce mouse embryonic stem cells to differentiate into many types of cells, including pancreatic beta cells, cardiomyocytes, and neurons. There is still only one reported study in which such differentiation has been shown to produce cells that have not been genetically altered and yet are able to correct a deficit after being transplanted.[4]

Our commentary, however, deals with another potential application for nuclear transfer treatment of infertility due to advanced maternal age. In vitro fertilization has given older women hope, but IVF runs up against a physiological limitation—aging eggs. No one undersands exactly what happens to eggs after several decades in the body, but doctors are convinced that old eggs are the key to age-related infertility. S. Wildman in New Yorker magazine[5] presents different options for older women regarding their infertility, including ovarian freezing and egg donations. The latter is a well-known procedure unacceptable to women who wish to have their own biological children. Ovarian freezing, although commercially available, remains very problematic. The problem is that eggs are more difficult to freeze than sperm or embryos. Because they are large cells filled with water, eggs are particularly vulnerable to the formation of ice crystals. Researchers have tinkered with the formula for decades: varying the concentration of cryoprotectant, the length of time an egg is exposed to it, the speed at which the egg is frozen and thawed. There were some successes, but none that could be duplicated consistently. Over the past 20 years, there have been only about 150 births from frozen eggs worldwide. Despite the recent successes in Italy, the American Society for Reproductive Medicine has labeled egg freezing "experimental" and strongly recommends that it be reserved for cancer patients whose fertility is in immediate jeopardy, and not be offered commercially to healthy women. Egg freezing costs $10,000 to $15,000 to harvest and freeze, then $500 annually for storage. The fate of frozen eggs and success rate of viable pregnancy remains questionable at best. Our limited experience with assessing egg aging suggests that cytoplasm is the most valuable component of the egg, while the nucleus is the most resistant.[6] Most of the research in this area had been conducted overseas, where the political climate appears more favorable.[6,7] Thus, J. Grifo from NYU, in collaboration with researchers in China, reported a triplet pregnancy after transfer of pronuclei from a patient's zygotes into zygote cytoplasts donated by a fertile woman. The work was done at Sun Yat Sen University Hospital, Guangzhou, China.

In the case following ICSI, 8 of 12 patient oocytes and 12 of 15 donor oocytes were fertilized. All pronuclei (PN) were then removed from each patient's zygotes and discarded. Male and female pronuclei were removed from each patient zygote and transferred into a donor cytoplast. Electrofusion of the patient karyoplast with the donor cytoplast resulted in 7 "reconstructed" zygotes. The 5 reconstructed zygotes that cleaved the 4 cell stage at 48h were transferred to the patient's uterus. Nuclear and cytoplasmic DNA profiles were analyzed in blood from the patient, oocyte donor and fetuses.Nuclear DNA fingerprinting was performed at 5 microsatellite loci, with subtraction of the husband's genotypes. Cytoplasmic mitochondrial DNA was analyzed by amplification and sequencing. A triplet pregnancy with fetal heartbeats was

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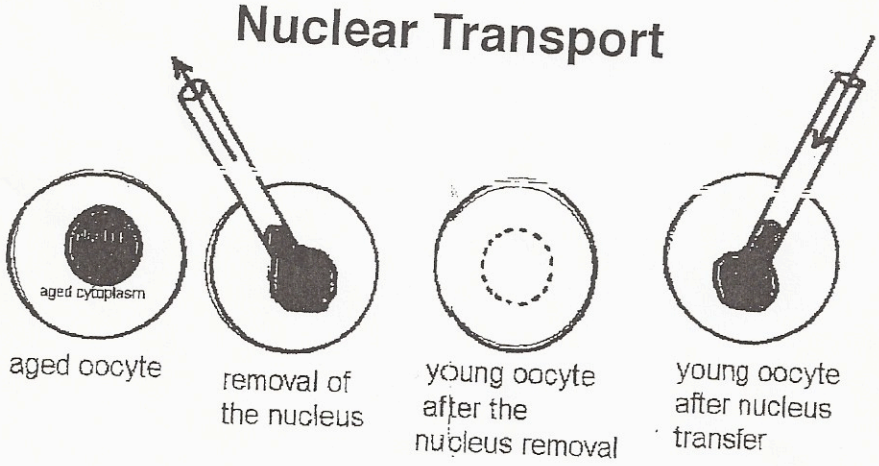

achieved. Fetal reduction to a twin pregnancy was performed transvaginally at 33 days post-transfer. At 24 weeks, fetus B delivered due to premature rupture of the membranes and died of respiratory distress. At 29 weeks fetus C delivered after intrauterine fetal demise due to cord prolapse. Normal karyotypes were found in the embryonic tissue (46, XY) and the 24 week (46, XX) and 29 week (46, XY) fetuses. Nuclear genetic fingerprinting confirmed that the nuclear DNA from 24 to 29 week fetuses matched that of the patient's. Thus, viable human pregnancies with normal karotype can be achieved through nuclear transfer. This finding suggests a unique approach to correct mitochondrial genetic disorders of maternal inheritance. Ongoing work to establish the efficacy and safety of nuclear transfer will result in its use as an aid for human reproduction (Fig 1).

We believe that this unique experience as well as one of the others will allow new opportunities for older women with poor cytoplasm and good nucleosis.

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