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William Burke

Patrick Pullicino

Edward J. Richard

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The Biological Basis of the Oocyte Assisted Reprogramming (OAR) Hypothesis: Is It an Ethical Procedure for Making Embryonic Stem Cells?

by

**William Burke, M.D., Ph.D., Patrick Pullicino, M.D., Ph.D.,
and The Rev. Edward J. Richard, MS, DThM, JD**

Dr. Burke is Professor of Neurology, Associate Professor in Medicine, and Associate Professor in Anatomy and Neurobiology at Saint Louis University Health Sciences Center. Dr. Pullicino is Professor of Clinical Neurosciences, Kent Institute of Medicine and Health Sciences, Canterbury, Kent, England. The Rev. Richard is Vice-Rector, Dean of Students and Associate Professor of Moral Theology, Kenrick Glennon Seminary, St. Louis, MO.

In response to the continuing controversy over embryonic stem cell (ESC) research, 35 ethicists and scientists have expressed their support for a new research proposal which they believe may yield pluripotent stem cells while circumventing the "creation" or destruction of human embryos. Their joint statement, issued June 20, 2005, is accessible on the Ethics and Public Policy Center web site:

www.eppc.org/publications/pubID.2374/pub_detail.asp

The joint statement was also published in *Origins*, the official publication of the US Bishops' Conference (7 July 2005, p.126ff).

This research proposal is called Oocyte Assisted Reprogramming (OAR), and the initial research would involve experiments with mice¹ but would proceed to human experiments if results look promising. OAR is a form of Altered Nuclear Transfer (ANT). Both OAR and ANT use a modified version of a procedure called somatic cell nuclear transfer (SCNT), previously named therapeutic cloning.

Embryo Development

In normal development a zygote cell divides into two cells called blastomeres. The cells remain totipotent for 2-3 days until the 4-8 cell stage when the embryo genome becomes activated.^{2,3,4} Up until this time, mRNA and proteins, deposited in the unfertilized egg by the mother's genome, control cell cleavage.⁵ These cells continue to divide such that at the 8 cell stage the developing human being is called a morula. At about 60-150 cells, a stage called the blastocyst is reached.^{3,6} The early developmental stages of the embryo appear devoted to cell cleavage and patterning which determines, through a gradient distribution of oocyte mRNA during cleavage,⁵ which part of the embryo will come to constitute its anterior-posterior and dorsal-ventral axes and right and left sides. The genome remains inactive and totipotent during these early stages of development.^{2,3,4} The blastocyst contains two types of cells⁶:

- 1) Embryonic stem cells (ESC) in the inner cell mass (ICM) of the blastocyst will develop into all tissues in the body of the human infant. These ICM cells are the type of cells used in ESC research. Normally ICM cells would become the epiblast, from which further stages of the embryo body-proper develop.
- 2) The second type of cell in the blastocyst is called trophoblast. These cells develop into extra embryonic tissues (e.g., the placenta or umbilical cord of the infant).

The Procedures

Somatic Cell Nuclear Transfer: In SCNT the nucleus of an oocyte (egg cell), containing the genetic instructions or genome, is removed. A somatic cell (e.g. skin cell) is then obtained from an adult animal or from an adult human and the nucleus containing the genome of the donor somatic cell is transferred into the egg cell. In a minority of cases, the egg cell "reprograms" the genome of the somatic cell such that the combination of egg cell plus somatic cell genome becomes a totipotent zygote.

If this is done with human cells and the zygote is implanted into a uterus, the zygote could develop into a fully grown human infant. In fact, according to Catholic Church teaching, the zygote itself is a human person.⁷

Altered Nuclear Transfer and Oocyte Assisted Reprogramming: In both ANT in general and OAR in particular, the donor somatic cell's genome is altered before it is transferred to the egg cell.

In ANT-1, an earlier version of ANT proposed by William Hurlbut, M.D., the alteration of the genome involves knocking out (i.e. removing) one of the 30,000 human genes in the donor cell genome that is required for the early development of the zygote (CDX2). In ANT-1, the new entity therefore develops to, but not past, the blastocyst stage due to the removal of the CDX2 gene, which is required for development of the trophoblasts.

The ANT-1 procedure has received harsh criticism from several scientists and Richard Doerflinger, an ethicist for the National Conference of Catholic Bishops,⁸ because it appeared to be the production of a genetically defective human embryo, rather than of a non-embryo. As Mr. Doerflinger said, "A short lived embryo is still an embryo."⁸ As a result of these criticisms, the ANT-1 procedure was abandoned as a proposal for research that could be ethically funded, and the OAR procedure was advanced in its place as a morally acceptable alternative. However, as we describe below, this ethical criticism of ANT-1 applies even more rigorously to OAR.

How Does OAR Differ From ANT-1?

The OAR procedure starts from the fact that although all cells in the human body contain the entire genetic code for a human being, not all genes are expressed in each cell. For instance, in a skin cell only the genes responsible for the characteristics of a skin cell are turned on.

The specific set of genes which are turned on or off in a particular cell type is the function of certain proteins in the cell called transcription factors (TRF). Other mechanisms, such as methylation of the genetic material, also play a role in regulation of gene expression.⁹ The transcription factors, which specify the cell type, bind to certain genes in the genome and turn the given gene either on or off.

It is at this point that the specific proposal of OAR begins. The proponents of OAR cite studies using a technique called immunocytochemistry to detect transcription factors that are thought to be absent in the zygote but present in the morula and in ES cells of the blastocyst. According to these studies, a transcription factor called "Nanog" is absent in the unfertilized mouse egg cell and in the 2-cell-stage embryo, but present in the morula and in higher amounts in 65% of ICM ES cells.^{10,11}

On the strength of this finding, OAR proponents hypothesize that Nanog is a pivotal transcription factor that is present only in pluripotent cells and not in totipotent cells.^{11,13} They stated: "Expression of these factors [transcription factors] therefore positively defines and distinguishes mere pluripotent cells from embryos."¹³ On this basis, they further hypothesize that Nanog is crucial for the development of a totipotent

zygote (human being) into a pluripotent cell. Furthermore, they claim they can use the presence or absence of Nanog to distinguish the totipotent single cell zygote from the morula and ES cells which they call puripotent.

Extrapolating from this hypothesis, OAR proponents propose to alter the donor cell genome by activating the gene producing Nanog before they transfer it into the enucleated egg cell (oocyte) and/or cause the egg cell to produce Nanog before the transfer. Scientists have discovered that the Nanog promoter has Oct4 and Sox2 binding sites, and that Oct4 and Sox2 activate or "turn on" Nanog expression.²⁰ Thus, the regulation of Nanog expression is not simple. In fact, both Oct4 and Sox2 transcription factors are present in the unfertilized oocyte long before Nanog is expressed in the morula stage of development.^{21,22} This suggests that the presence of these transcription factors alone is not enough to activate Nanog. According to their hypothesis this early expression of Nanog suffices to ensure that the transfer of this altered donor cell genome into the enucleated egg cell will directly produce a pluripotent stem cell and not a totipotent zygote (i.e. human being), as critics of ANT-1 thought that that procedure would have done.

This hypothesis accordingly provides the basis for the judgment that a totipotent zygote is never present in OAR-produced cells and that no totipotent zygote precedes the formation of so-called pluripotent cells. This is also the ground of the ethical judgment that OAR is a morally acceptable procedure involving no creation/destruction of human embryos.

The Ethical Problems

There are, however, serious problems with this hypothesis which prevent the moral certainty required to determine that OAR overcomes the principal ethical dilemma involved in ANT-1, namely that the procedure produces a short lived embryo. Examples follow:

1. The oocyte (egg cell) is a very powerful reprogramming cell itself. The enucleated oocyte can reprogram a skin cell genome, with all of its specifying factors including transcription factors and gene methylation, into totipotency in a matter of hours.⁹ We have Dolly the sheep and other cloned animals as proof of this fact. Therefore, the OAR proponents' claim that just one more transcription factor, i.e., Nanog, will prevent the oocyte from reprogramming the donor cell to totipotency seems doubtful.

Proponents claim that the oocyte can be manipulated to express genes which would activate expression of mRNAs for transcription factors.^{12,14} However this is not sufficient. To change one cell type to a different cell type, the mRNA needs to be translated into the specific transcription factor protein; the transcription factor protein needs to be

processed and transported to the nucleus so it can attach to its target genes. Then the target genes need to be transcribed, translated and processed into a functional protein before a claim that the cell type has changed can be made. The fact that two transcription factors, Oct4 and Sox2, which activate the Nanog gene are present in the unfertilized oocyte^{21,22} but Nanog is reported to appear first in the morula stage,²⁰ indicates that any genetic manipulations in the donor genes or in the oocyte will not be enough to activate expression of Nanog. This may be due to the fact that the oocyte cytoplasm removes all factors which would restrict the totipotency of the zygote.

However, in the OAR entity there are at least two mechanisms which will prevent this cell type change from occurring immediately. First, all specifying factors are removed rapidly from the donor cell genome by the oocyte cytoplasm⁹ to make a totipotent embryo. Secondly, the donor cell genome does not become active in directing protein synthesis and the development of the cell until day 2-3.^{2,3,4} For instance, Tong et al. showed that human cells are totipotent until at least the four cell stage when RNA synthesis starts.² Therefore, the new entity will share the normal human developmental path until at least this time.

Further, OAR proponents must demonstrate that the protein products of the target genes prevent the initial development of a zygote.⁸ If these products act at a later stage of development, a short lived embryo will still have been produced by the procedure.

2. Nanog keeps the cell, in which it is present, in the undifferentiated state. The most undifferentiated state is totipotency. It is therefore a leap to conclude that Nanog's presence necessarily overcomes totipotency to produce only pluripotency.

The fact is that all that the data actually show is that Nanog keeps ES cells in an undifferentiated state. Thus, the presence of Nanog is perfectly compatible with the interpretation that OAR creates a zygote incapable of differentiating past the epiblast stage of development. Therefore, OAR, like ANT-1, is arguably the production of a crippled embryo incapable of fully developing into a human infant.

3. The term pluripotent used to describe morula and ICM ES cells is ambiguous and not used with the same meaning by all scientists. For instance, John Shea, M.D., of Toronto, states, "All cells of the early human embryo are totipotent until shortly after the blastocyst stage."¹⁵ This same view is described in the testimony of David A. Prentice to the United States Senate.⁴ Further, if cultured ES cells are experimentally substituted for ES cells in the blastocyst, these cells can contribute to any tissue including the germ line. "Thus these cells are totipotent."¹⁹ The only cells which so-called pluripotent ES cells cannot form are the trophoblasts, which produce extraembryonic tissues, like the placenta, which are not essential parts of

the human body. Calling ES cells pluripotent instead of totipotent appears in this respect to be a distinction without a morally relevant difference.

In fact, because Nanog is present in morula cells which develop to the blastocyst stage, we must conclude that it cannot prevent these cells from forming the entire blastocyst including the trophoblasts. Therefore, Nanog cannot be reasonably expected to prevent the new entity from forming the entire blastocyst including trophoblasts. Thus the new entity, even in the presence of Nanog, is totipotent in the strictest sense of this word.

4. OAR proponents dispute this conclusion, stating that an experiment manipulating Nanog has not yet been done.¹⁴ We disagree.¹⁶ First, the critical experiment, showing that Nanog appears in morula cells before the trophoblast cells are formed, has already been done in mice.¹¹ Secondly, Nanog measurement was not reported in the single cell zygote and was absent in 35% of mouse ES cells and a certain fraction of human ES cells.¹¹ These findings raise questions about the claim that Nanog is a marker of pluripotency. Thirdly, experiments to determine presence or absence of Nanog in a human zygote would not be ethical because they would require destruction of the zygote.¹⁷

5. Perhaps the most dangerous ethical leap made by OAR proponents is their confidence that science, by itself, can answer the critical ethical question: Does the zygote exist even for a short time, during the OAR procedure? Proponents claim this is only a technical question and that experiments on mice will give us the answer. If these experiments support their hypothesis, they will be able to go on to similar human experiments because there is supposedly no moral issue left to be determined.

However, there are limits to certainty in biology. For instance, the claim that Nanog is not detected in the 2-cell-stage embryo, using a specific biological procedure, is not the same as certainty that it is absent in the single cell zygote. In fact, results measuring Nanog in the single cell zygote were not reported in the study OAR proponents cite to support their claim that Nanog is an essential pluripotency marker.¹¹ In addition, the same study showed that Nanog was absent in 35% of mouse ES cells and in a certain fraction of human ES cells.¹¹ Due to the limited statistical and methodological certainty allowed by biological science, the occurrence of technical errors in biological experiments, the difference between human and animal embryo development, the rapidity by which the cloning procedure produces a totipotent zygote, there is no biological experiment that will prove with certainty that a human zygote never exists during the OAR procedure.

In testimony before the United States Senate, Dr. George Q. Daley, a preeminent stem cell researcher, stated: "Even if this strategy [OAR] works in mice, there is no guarantee it will work in humans, and verification would require the creation and destruction of many

manipulated human embryos, which might or might not have the altered characteristics that would make using human oocytes ethically acceptable."¹⁷ OAR experiments using human oocytes would require a control group for comparison to the OAR group. The control group would be normal human zygotes (i.e. human beings) which would be destroyed during the experiment.

Therefore, one cannot ethically perform the experiments that are needed to provide the certainty required to answer the critical moral question (i.e., Does a human being ever exist during the OAR procedure?) As Dr. Daley put it: "I am not sure you could ever satisfy the critics. They could say 'Well you might get it to work but how often does it fail?'"¹⁷ Let us hope that a mistaken judgment by scientists based on the claim that OAR works in mice will not lead the Church to view the creation of crippled human embryos for research positively.

6. Finally, there is a broader ethical problem with OAR. As with in vitro fertilization, the OAR procedure would take a component of human reproduction, the oocyte or egg cell. OAR then uses it in what is essentially a cloning procedure normally designed to produce an independent living entity, a procedure that would therefore otherwise be immoral if used on humans. Given the evidence presented above, it seems unfounded to conclude that activating or blocking the expression of a gene in this cloning procedure is sufficient to prevent it from being cloning or to alter the human nature of the entity it creates.

We do not think a similar alteration in the genetic material used for in vitro fertilization would make the process of in vitro fertilization moral. Similarly, we do not think this alteration in cloning makes it any more morally acceptable. A combination of wrongs cannot make the end result good. Proponents argue that OAR is not cloning because they do not intend to create an embryo.¹⁴ However this begs the critical question because the SCNT procedure, used in OAR, is by definition cloning.

A simple analogy may help illustrate the essential point that neither Altered Nuclear Transfer in general nor Oocyte Assisted Reprogramming in particular can truly bypass the production of a zygote, which, if human cells are used, is a human being at its earliest stage of development.

Let's consider that the zygote is a complete book containing 30,000 pages, one page for each gene in the genome. If you remove a single page – let's say, page 61, for the gene that directs development of the embryo's trophoblast cells at the blastocyst stage, as in ANT-1 – would you have created something that is not a book (human person)? Or would you call it a defective book (crippled embryo)?

Carrying this analogy one step further: If you also add a new page 200 (that is, insert a gene which blocks differentiation of the embryo past

the epiblast stage, as in OAR), would you have an entirely new thing or would you have a defective book (crippled embryo)?

The answer in both cases is obviously that you would have a defective book (crippled embryo).

There are other problems with the OAR hypothesis having to do with the overarching and complex moral and ethical questions that surround these procedures that would involve "harvesting" from women the egg cells that would be required for the purpose of producing the desired stem cells. But the arguments we have presented here should be enough to give pause as to the ethical validity of OAR.

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