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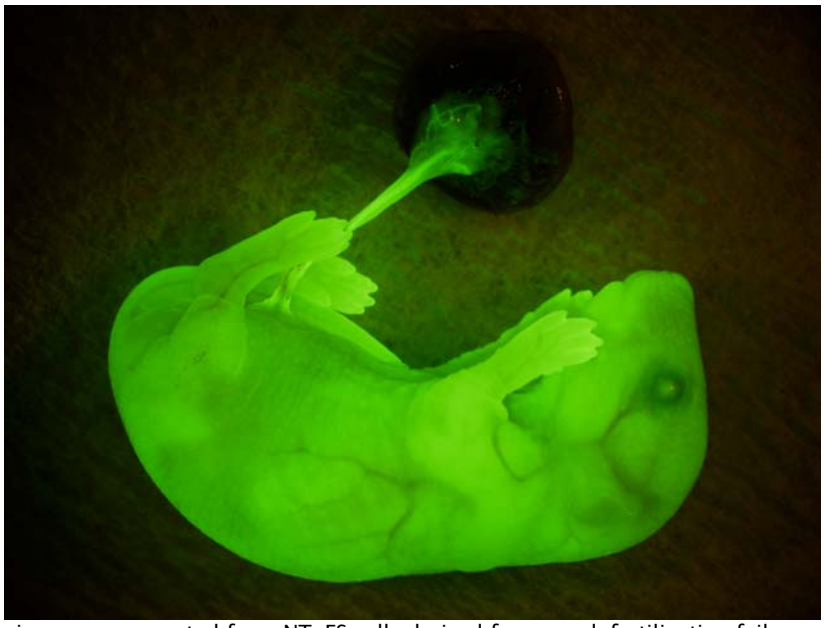
New source of oocytes for SCNT?

February 21, 2007 – The study of human embryonic stem (ES) cells has enjoyed much attention for the promise these cells hold as a potentially limitless source of cells for use in drug development and regenerative medicine, but the field has also been dogged by controversy arising from a number of ethical issues. One of the most hotly debated of these involves how oocytes used in ES cell derivation by somatic cell nuclear transfer (SCNT; also known as therapeutic cloning) are obtained. Conventional wisdom has it that only "fresh" oocytes can be used, but new research from the CDB Laboratory for Genomic Reprogramming (Teruhiko Wakayama; Team Leader) suggests that even oocytes that fail to fertilize may be usable in SCNT. If it can be shown that the same holds true for human egg cells, this finding could help to resolve many issues surrounding oocyte donation, as large numbers of such oocytes are simply discarded in IVF clinics.



Chimeric mouse generated from NT-ES cells derived from aged, fertilization failure oocyte.
The donor nuclei were from mice engineered to express GFP, which can be seen as a green glow in all tissues.

The Wakayama lab collected oocytes that had failed to fertilize after insemination, which they named "aged, fertilization failure" (AFF) oocytes, and used them as recipient eggs for nuclear transfer. The donor nuclei used in these experiments were taken from various strains of lab mice, including one engineered to express the GFP protein ubiquitously, causing the animals to glow green under fluorescent light. While the efficiency of nuclear transfer and subsequent early development was lower for the AFF oocytes, they found that embryos that did reach the blastocyst stage could be used to derive ES cells at a success rate equivalent to that when starting from fresh oocytes. "We're hoping that this method can be optimized for use with human oocytes, which would open up a substantial new source of oocytes for use in some kinds of research and overcome a number of the objections that have been voiced over this promising technology," notes Wakayama.

The cell lines established from the fertilization-failure oocytes expressed the molecular hallmarks of pluripotent cells, such as Oct3/4 and Nanog, and all cells examined were of normal karyotype (meaning their chromosomes were in the appropriate number and arrangement). Even more convincingly, these nuclear

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transfer ES cells contributed to the development of healthy, fertile offspring, with functional differentiation into both germline and all somatic germ layers.

In full-term cloning and intracytoplasmic sperm injection (ICSI) experiments, the AFF oocytes proved to be less amenable than their fresh counterparts, which the authors reckoned might be attributable to defects in genomic reprogramming. A partial deficiency in this mechanism could account for the fertilization-failure egg cells' ability to generate nuclear transfer ES cells, but not to support full-term development. This subtle but crucial difference could help to answer many of the standing criticisms about the trade-offs involved in dismantling a blastocyst-stage embryo in order to derive ES cells for research, for if the blastocyst is naturally destined never to reach later stages of embryonic development, arguments regarding the sacrifice of individual potential largely become moot. Similar concepts underlie a number of other methods, sometimes referred to as altered nuclear transfer, in which the embryo is genetically engineered to prevent its development into an individual, while allowing it to grow to the blastocyst stage.

"What's important about this demonstration is that it shows that even aged oocytes that failed to fertilize on an initial attempt can be used to generate ES cells, which are extremely useful in basic research and show great future promise in medical applications," says Wakayama. "While past attempts to use fertilization-failed human oocytes have been unsuccessful, we hope that this work shows that that may have been due to the nuclear transfer technique, rather than any inherent biological deficiency."

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