Scintillating advances on somatic cell nuclear transfer and the implications for human ARTs

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2013 continues to be a year of breakthroughs in the field of reproductive medicine and biology. Yet another of these has just appeared in the journal *Cell*.

Not since the pioneering efforts of Ian Wilmut and the late Keith Campbell put Dolly on the map has there been a measurable success in somatic cell nuclear transfer (SCNT), an advance capable of melding the available technology with the growing and pressing need to generate “patient specific” stem cells for drug screening, disease modeling, or potential therapeutic applications. In fact, it is fair to say that while SCNT can yield live young in some mammalian species, and especially those of agricultural and bioreactor value, the road to achieving any sort of efficiency for the production of quality embryos and derivative stem cells from primates (both nonhuman and hominid varieties) has been fraught with setbacks. And just as the collective field of human ARTs has taken aim at the matter of gamete and embryo quality in maintaining the safety and efficiency standards marking treatments for infertility, so too in parallel array has the domain now referred to as Regenerative Medicine waited not so patiently for a breakthrough that would set a course well beyond what induced pluripotent stem cell (iPS) technology has brought to the table.

So what makes the latest work so scintillating? As reported in *Cell* on May 15, 2013 (Tachibana et. al., Human embryonic stem cells derived by somatic cell nuclear transfer, <http://dx.doi.org/10.1016/j.cell.2013.05.006>), Shoukhrat Mitalipov and their colleagues at the Oregon Health and Science University and Oregon National Primate Research Center (ONPRC) segued their program on SCNT in primates to that of humans. They achieved this by keeping a keen focus on the

qualities of oocytes retrieved after COH, that would pass muster through the perilous pathway of the egg cell cycle and activation coda that etches the conceptus ever so close to successful implantation. What defines this work in physiological terms is not only the elegance with which this group moved cautiously and carefully from the rhesus macaque animal model to the human, but the attention to detail brought to bear on the biology of the oocyte as it pertains to the impact of human ART technology. As described below, taking the time to tease apart this piece of technological bravado will have immediate merits for the readership of JARG willing to envision the future of human ARTs.

As alluded to above, central to obtaining sufficient numbers of oocytes for embryo production is the process of COH. While long held to be the gateway to the “more is better” mentality that fuels human ARTs, the growing awareness that “less is better” when it comes to COH resonates conspicuously in the work of Tachibana and colleagues. Rather than looking to the normative outputs of pregnancy rates and outcomes, the drive to produce ES cells from SCNT embryos was assayed within and between young egg donors using stimulation protocols that would result in varying numbers of oocytes retrieved. While it will come as no surprise that GnRH agonist protocols yielded on average more eggs per retrieval than those receiving antagonist, when assayed for embryo development after SCNT, significantly higher rates of compacted morulae and blastocysts were obtained in the group receiving antagonist. Moreover, when blastocysts from either group were evaluated for their ability to generate stem cells, the antagonist group alone resulted in viable stem cell lines to the tune of 35 % efficiency compared to 0 % for eggs derived from agonist cycles. When one superimposes the findings that both of these outputs—embryo development and stem cell derivation—were significantly improved when fewer (<10) rather than more (11–15 and >16) oocytes were retrieved, the implication that “less is better” (using moderated stimulation cycles) is reiteratively reinforced when it comes to the matter of embryo quality! So much for the lessons learned from COH.

Capsule A remarkable accomplishment in the field of stem cell research uncovers novel attributes of oocyte quality that will impact the field of human ARTs in the near future.

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What about the process of making SCNT embryos, in this case for the strict purpose of stem cell derivation? As a somewhat casual observer of the SCNT technology for over 15 years, and an obsessive life-long learner and student of oocyte biology, take my word for it when I suggest that biology trumped technology in the course of these elegant and insightful experiments! No less than three major advances are resident within this paper, involving the refinement of techniques required to achieve some semblance of efficiency when it comes to SCNT embryo production.

First and foremost is the issue of removing the metaphase-2 spindle, referred to as enucleation to indicate the extraction of the host cell (oocyte) nuclear (but not mitochondrial) genome. Both physical and chemical enucleation methods have been used successfully in past animal studies, but the parallel benefits to the embryo accorded by retention of cell cycle factors on chromosomes and spindle microtubules prior to nuclear transfer and egg activation have only more recently been appreciated. The major obstacle addressed and overcome in this study is founded on the problem of how best to synchronize the meiotic cell cycle state of the oocyte with that of an incoming interphase genome from the donor cell. The confluence of traditional enucleation treatments with a mindset aimed at maintenance of an endogenous reprogramming and chromatin remodeling requirement and virus-mediated delivery/fusion (the second of their revamped conditions tested initially on primate oocytes) brought not only the immediate reward of better embryo development, but also opened the door for improved stem cell derivation.

The third and final advance in SCNT technology had to do with obtaining robust activation of the oocyte cytoplasm, such that the oocyte would commit to a pathway of initial development carrying the somatic cell genome it inherited by donor cell fusion. Again, the process of trial and error yielded results from primate oocyte investigations that were telling and pace setting when it came to getting human SCNT embryos on their merry way, without having to dote over the many things that make sperm so special at the time of fertilization. Here the secrets resided in giving the oocytes an extra activation signal in the form of electroporation alongside the conventional use of the calcium ionophore ionomycin. Finessing cytoplasm activation with this extra kick was sufficient to again improve developmental rates and stem cell derivation, and will in the end have many a “SCNTologist” kicking themselves for not having put these pieces of the puzzle together in an earlier cloning era.

In the end, what emerges from the work of Mitalipov and colleagues is an earnest and measured effort to achieve the goal of reliably and safely producing “patient specific” stem cells from human SCNT embryos for the benefit and treatment of human disease. In the short term, there is a much less altruistic, but nonetheless pressing, set of issues that will necessitate a very close look at the ethical implications revived with this work in the context of therapeutic cloning. While these matters must remain front and center as the discourse unravels itself, the field of human ARTs should take special note of the lessons this seminal study will leave for us to cogitate for the treatment of infertility in the not so distant future.