Reproductive medicine 2013: The year in review

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As exciting as 2012 was for the disciplines of reproductive biology and medicine, gaining traction within and without expected destinies for basic science and clinical advances, 2013 has far outshone it for several reasons. Not the least of these is the fact that some of the clinical areas receiving attention of late—but remaining not-so comfortably on the fringe of translation—have now taken a quantal leap into the realm of legitimate application. As discussed below, 2013 saw witness to breakthrough studies that bring hope for the use of patient-specific stem cells in the field of regenerative medicine and will inform the practice of fertility preservation with impending applicability for men and women alike. A common theme for the advances realized during 2013 is the legitimization of the “from bench to bedside” paradigm—the highly touted but difficult to demonstrate mantra of translational medicine. Exemplative of this was the work reviewed last month in JARG illustrating the translational value obtained from basic science studies in mice within the context of primary ovarian insufficiency (POI) in humans (see below). Moreover, the insights gathered on the origins of germ cells and their interconvertibility with stem cells have placed this promising area of study on firm footing and with molecular details that will foster deeper understanding of the epigenetic mechanisms at work in reproduction. The reader should bear in mind that the menu served below reflects the author's bias and enthusiasm in a manner consonant with the goals and objectives of JARG. If nothing else, it is hoped that this short

passage through the year 2013 will give our readers a chance to expand their own senses of curiosity and criticism as we move forward in the field of reproductive medicine.

Starting off our list of notables for this year are two papers that appeared in parallel in *Nature* early in 2013 that have taken the concept of genetic tinkering to a new and one might say “nanosurgical” level. Evading genetic risks posed by the contribution of mutated and disease-provoking mitochondrial genomes is an intrinsically challenging task since such genomes are invariably and robustly one of Mother Nature's irreconcilable gifts to an incipient zygote. To circumvent this problem, the laboratories of Mitalopov and Egli lead efforts to produce human blastocysts with stem cell generating capabilities by performing organellar transplants—namely the spindle complex bearing the nuclear genome—such that zygotes were produced with a distinct and unique mitochondrial genome (Tachibana, M., et al., 2013 Towards germline gene therapy of inherited mitochondrial diseases. 493:627–631; Paull, D., et al., 2013 Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. 493: 632–637). While the technological nuances between the studies showed variations on a common theme, the importance of oocyte quality factors were judiciously in play on the road to producing viable embryos for purposes of stem cell generation.

With the momentum growing in the field of Developmental Origins of Adult Health and Disease (DOAHD) and ushering in a level of awareness regarding the importance of pre-and peri-conceptual health, the next two studies tackle the importance of the epigenetic landscape during development from the epidemiological to molecular levels. First, from the Finnish national registry studies comes the recognition that pre-conceptual use of folic acid may have a measurable impact by reducing offspring risks for developing autism spectrum disorders (Sure'n, P. et al., Association between maternal use of folic acid supplements and risk of autism spectrum

Capsule 2013 has been a remarkable year for advances in reproductive biology and medicine with striking examples of how the uncovering of fundamental mechanisms gleaned from animal studies have paved the way for unanticipated gains of clinical import. A selection of papers from 2013 are reviewed.

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disorders in children. *J. American Medical Association* 309:570–577, 2013). In *Cell* recently, a striking study appeared that takes the specific issue of folate metabolism to a tangible and cogent level by demonstrating that in mice, mutations in the enzymes responsible for folate metabolism result in specific epigenetic alterations in mice (Padmanabahn, N. et al., Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell* 155:81–93, 2013). Even more evocative, or in fact alarming, is the finding that transgenerational disease outcomes find their roots in epigenetic changes imposed in the germ line of grandparents of affected offspring. Without the availability of technology to study these processes through several generations, as is made possible by the experimentally tractable mouse model, findings of this kind would have been difficult, if not impossible, to be made in other commonly used animal models.

Fertility preservation matured as a discipline in human ARTs with the publication of two papers this past year. On the male side, the troubling confounder of cancer cell contamination when using tissue, or in this case stem cell, transplantation to restore fertility received critical attention in a report from the Orwig laboratory at the University of Pittsburgh (Dovey, S., Valli, H. et al., Eliminating malignant contamination from therapeutic human spermatological stem cells. 2013. *J. Clin Invest* 123: 1833–1843. Doi:10.1172/JCI65822). And on the female side, promising results from a study on rodents using the drug AS101 indicate that adjuvant therapies may minimize follicle depletion effects of commonly used chemotherapeutics like cyclophosphamide (Kalich-Philosoph, L., et al., Cyclophosphamide triggers follicle activation and “burnout”; AS101 prevents follicle loss and preserves fertility. 2013. *Sci. Transl. Med.* 5:185ra62. Doi:10.1126/scitranslmed.3005402.). The genetic health and well-being of human germ cells has historically assumed center stage as a concern following DNA-damaging therapies and the impact of advancing age on genomic integrity in the germ line.

That maternal age contributes to the loss of genetic stability in human oocytes is well known. But, to date, most studies have focused on alterations that exacerbate the incidence of aneuploidy, emphasizing the loss of cell cycle checkpoints or impairments in spindle or chromosome functionality. With the publication of studies on the BRCA1 gene in mouse and human oocytes from the laboratory of Oktay, a novel and insightful change in direction with clear clinical implications has been suggested (Titus, S., et al., Impairment of BRCA1-related DNA double strand break repair leads to ovarian aging in mice and humans. 2013 *Sci Transl Med* 5: 172ra21. DOI: 10.1126/scitranslmed.3004925). This work identifies a central role for the DNA damage recognition and repair genes in the maintenance of genetic integrity of mammalian oocytes and demonstrates that the progressive loss of follicles observed

upon ovarian aging is likely a consequence of an impaired repair system that results in the accumulation of DNA double strand breaks.

Recognizing the desperate situation that patients with primary ovarian insufficiency find themselves in, the work published recently from the Kawamura and Hsueh laboratories is recognized for revealing basic mechanisms that drive the activation and continued development of the ovarian follicle and putting these fundamental findings into a clinical context (Kawamura, K. et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. 2013. *Proc. Natl Acad. Sci. USA* www.pnas.org/cgi/doi/10.1073/pnas.1312830110). The fact that they were able to extend bench observations on rodents to a sophisticated treatment scenario that resulted in the reactivation of ovarian activity capable of providing quality oocytes for embryo production is a laudable accomplishment in its own right. The fact that this procedure resulted in a live birth using vitrified human ovarian cortex lends further evidence to the extraordinary nature of this work and the hope it will raise for POI patients in the future.

The wonderful world of stem cell biology has been witness to a remarkable series of discoveries primarily drawing on the use of animal models. The birthing of germ cells has until now eluded description even in the face of a series of papers that have compellingly shown that ESCs or iPSCs can be coaxed down the pathway of gametogenesis in mice under the right conditions. Making a gamete from a stem cell is well within the purview of basic science. Making a stem cell from the unionized egg and sperm at fertilization is a fact of Nature! How the latter is accomplished at a mechanistic level was unknown until now.

As we have highlighted before, the Saito laboratory from Kyoto, Japan, has once again added an important chapter to the matter of plasticity as it pertains to the interconvertibility of stem cells and gametes. With their past accomplishments defining the in vitro conditions and transcription factor (TF) requirements needed to transform ESCs or iPSCs in mice to that of card-carrying eggs or sperm, the discriminating character that would improve the efficiency of this process has now been rigorously elaborated upon in their latest paper (Nakaki, F., et al., 2013 Induction of mouse germ-cell fate by transcription factors in vitro. *Nature* :501:222–226 doi: 10.1038/nature12417). Coupled with the report from the Surani laboratory where a similar mechanism was uncovered, our understanding of how the decision to follow germ line or stem cell lineages is made has been sharply defined in molecular terms (Magnusdottir, E., et al., 2013. A tripartite transcription factor network regulates primordial germ cell specification in mice. *Nature Cell Biology*, 15:905–915, doi:10.1038/ncb2798). The pathway to these discoveries necessitated separating the TF needs that would bypass transiting through a mesodermal program thus allowing for direct transformation of epiblast-like cells

into a primordial germ cell-like state! Both of these studies open the door for explorations of mammalian gametogenesis with wide-ranging implications for basic and clinical applications.

Carrying on with the theme of gametogenesis and stem cells and the roots of acquiring a complexion in the likes of one or another, efforts this past year have also uncovered many of the secrets behind the epigenetic gymnastics underscoring cell fate decisions. Among these, a series of papers appeared probing the timing of changes in the methylome with respect to the erasure of chromatin marks that set the stage for building the next generation of gametes. Growing awareness of the role of Tet genes identified how the Tet1 and Tet2 genes elicit the epigenetic starting line once genome-wide demethylation has been completed. (Vincent, JJ., et al. 2013 Stage-specific roles for tet 1 and tet2 in DNA demethylation in primordial germ cells. *Cell Stem Cell* 12:470–478 doi: [10.1016/j.stem.2013.01.016](https://doi.org/10.1016/j.stem.2013.01.016)). And in a paper from the Zhang laboratory at Harvard Medical School, the underpinnings for staying the course of epigenetic reprogramming if you are female, but restoring the stemness required for spermatogenesis if you are a male, were defined again as a function of previously unknown chromatin modifying states (Yamaguchi, S. et al., Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. 2013 *Nature* 501:222–226. doi: [10.1038/nature12417](https://doi.org/10.1038/nature12417)). And in a final scene from the script on germ cell plasticity, details on the performance enacted by germ cells as they transit from a differentiated to totipotent state at the time of fertilization have emerged. Published in *Cell* this past year (Lesch, B.J. et al., A set of genes critical to development is epigenetically poised in mouse germ cells from fetal stages through completion of meiosis, 2013, *Cell*

110:16061–16066 <http://www.pnas.org/content/110/40/16061>) a group of developmentally important genes were identified that retain an epigenetically “poised” state throughout gonadogenesis and meiosis in males and females reserving the right to activate and produce a transcriptome that renders totipotency to the conceptus.

And finally, in a paper symbolizing the critical link between human ARTs and the field of regenerative medicine, is the work from the Mitalopov group demonstrating for the first time that ESCs can be derived from human oocytes using SCNT technology (Tachibana et al., Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer, *Cell* 153, 1–11, 2013 <http://dx.doi.org/10.1016/j.cell.2013.05.006>). Following their earlier pace-setting studies using macaques, which indicated that oocyte quality was a rate-limiting determinant for the derivation of pluripotent stem cells, this work not only paves the way for production of patient specific ESCs from somatic cell nuclei but guides and informs the current practices of egg retrieval used in the field of human ARTs.

From this collection of publications comes a brief glimpse of the inroads being made into the fields of regenerative and reproductive medicine. As 2013 draws to a close, there is refreshing sense of accomplishment illustrated in the content of the works cited above. But with any sense of optimism for where this work is going must also come pause as the ethical, legal and religious implications of advances like this take form especially in the context of treating human disease. The passing of Bob Edwards this year should forever be a reminder that with each step he took along the road to today’s practice of human ARTs, came pause, deliberation and dedication to his belief that humankind would benefit from scientific progress.