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www.publish.csiro.au/journals/rfd *Reproduction, Fertility and Development*, 2006, **18**, iii

Introduction to the symposium on 'Non-Human Primate ART to ES Cells'

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First, I want to thank Barry Bavister and Carol Brenner for organising this meeting. Under the circumstances existing in the wake of Hurricane Katrina, it was nothing short of heroic, from my perspective.

I will begin by providing a little foundation for the symposium today. Two years ago at the International Embryo Transfer Society meeting held in Portland, Oregon, Richard Stouffer, Mary Zelinski-Wooten and I organised a symposium entitled 'ARTS in Action in Non-Human Primates'. We had three broad objectives in mind: (*1*) to review the current state of reproductive research employing the assisted reproductive technologies (ARTs) in non-human primates (NHP); (*2*) to enhance interactions and collaborative efforts within and between the National Primate Center system and academic institutions concerning these technologies and reproductive research; and (*3*) to identify and discuss new developments in reproductive physiology, gamete/embryo biology and efforts to exploit the ARTs in non-human primates (Wolf 2004). Individual topics included gametogenesis, gametes and fertilisation, implantation and pregnancy, ART applications – for instance, in the production of animals with desired genotypes, contraception, nuclear transfer, twinning – and finally, at the bottom of the list, was embryonic stem (ES) cells. Given the present focus on ES cells, it is clear that over the last 2 years we have moved ES cells from the bottom of the list to the top. Conclusions drawn by Dr Bavister at the end of the symposium in 2004 were, first of all, that major technical problems associated with routine production of non-human primates using either conventional *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) are largely solved. Research activities in non-human primates that might carry a translational component include developing chemically defined culture media for embryo development, establishing non-invasive markers for oocyte and embryo quality and perhaps oocyte *in vitro* maturation. Additionally, unique approaches that could and should be applied strictly to non-human primates include strategies to produce identical twins, either by blastomere splitting or blastocyst bisection, production of transgenic animals, and, of course, somatic cell nuclear transfer to produce cloned animals. Finally, the caveat that the ARTs in non-human primates could contribute to stem cell biology and technology was mentioned.

The objectives of today's symposium are: (*1*) to educate investigators, the speakers as well as the audience, about the challenges and successes associated with non-human primate ES cell biology; and (*2*) to foster communication relevant to the use of non-human primate ES cells in translational research. By way of a historical perspective, primate ES cells were first isolated in the rhesus monkey by Jamie Thomson's group in Wisconsin, from embryos that were produced *in vivo* and recovered from the uterus by flushing. Such cells, isolated from *in vivo*-produced embryos, should serve as our 'gold standard' for assessing primate ES cell lines. At the National Primate Research Center in Oregon, we have established additional rhesus monkey ES cell lines using either ICSI-derived or IVF-produced embryos.

The common marmoset was the second non-human primate species from which embryonic stem cells were derived, again by Jamie Thomson's group. There is, however, a recent report from Sasaki and co-workers (2005) in Japan, describing their efforts to produce marmoset ES cell banks. In the cynomolgus macaque, Suemori and co-workers (2001) have isolated four ES cell lines and Vrana and co-workers (2003) produced a single ES cell line from a parthenote. With regard to the characteristics of non-human primate ES cells, I'm sure most of you are aware of their growth and maintenance properties that resemble human ES cells as opposed to ES cells from the mouse. As an addendum to these introductory remarks, I will provide a brief overview of our studies on rhesus monkey ES cells at the Oregon National Primate Research Center.

We have isolated over 20 ES cell lines from embryos produced by application of ARTs. Three of these lines showed stable but abnormal karyotypes and we observed an even distribution between X- and Y-bearing lines. In characterising these lines, of course, we used conventional approaches of detecting pluripotent genes and gene products, and specific markers such as SSEA 3 and SSEA 4 by transcriptional analysis or indirect immunocytochemistry. We are beginning to ask questions about the heterogeneity of ES cells as there is substantial evidence that lines do vary. One of the ways of doing

 10.1071/RDv18n8_IN 1031-3613/06/080iii

this is to look at the pluripotency of cell lines during directed differentiation *in vitro*. Briefly, when ES cells are removed from their feeder layers, they spontaneously differentiate into embryoid bodies containing cells representative of the three major germ layers: endoderm, ectoderm and mesoderm. We can subsequently drive the cells in embryoid bodies into a population of progenitors in the presence of FGF2. Upon withdrawal of the FGF2, spontaneous differentiation produces cells found in the central nervous system, the most prevalent of which are serotonergic neuronal phenotypes. We have been able to produce populations representing the ectodermal lineage comprising ~90% serotonergic neuronal phenotypes. Interestingly, for endodermal lineage differentiation, a similar protocol is used to produce populations of progenitor cells. We then add exenden-4 and nicotinamide to induce differentiation into a lineage that is predominantly pancreatic β cells. Using this protocol, we have recovered populations of C-peptide and insulin-positive cells that represent ~65–70% of the total population. By quantitating the ability of each ES cell line to differentiate into specific phenotypes using this strategy, we can assess line-specific differences.

We have begun to define the gene expression profile in monkey ES cells using the recently released Affymetrix gene chip that was developed in conjunction with Drs Robert Norgren and Eliot Spindel. The array contains over 50 000 probe sets recognising over 20 000 genes spanning the entire genome. We began by asking the question 'What is the variability within a culture of embryonic stem cells?' To our relief, we found only a 1–2% difference in the transcriptomes among colonies within a single culture. Next we asked 'What is the interline variability?' We compared five different monkey ES cell lines against each other and against a somatic cell control of fibroblasts. The variability between ES cell lines was between 6 and 13% but when compared with fibroblasts, transcriptome differences were on the order of 25 or 26%. If we restrict our attention to expression changes that are at least two-fold or greater in magnitude, interline differences are on the order of 4%, with an 18% difference when we compare ES cell lines with the fibroblast control. From this humble beginning, we conclude that ES cell lines are not identical but are more similar to each other than to a differentiated cell. We have now begun the process of defining pluripotent or 'stemness' gene candidates and comparing results from the monkey with those available from the mouse and human (J. Byrne, S. Mitalipov, L. Clepper and D. Wolf, unpublished data).

In future studies, we will continue to address intra- and inter-line variability in monkey ES cells. We have also proposed the creation of xeno-free banks of MHC-typed ES cell lines in preparation for transplantation studies and a definition of the requirements for ES cell-derived phenotype survival and function *in vivo*.

With these introductory remarks, I will now end and we can move on to the formal presentations.

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