Focus on stem cells in reproduction

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Stem cells have long been of interest to biologists interested in reproductive systems, with the spermatogonial stem cell providing one of the best understood systems and niches in mammals *in vivo*. Although highly controversial, experiments which could be interpreted as indicating the existence of ovarian stem cells capable of regenerating the oocyte pool postnatally (Johnson *et al.* 2004), have also rekindled interest in the search for stem cells in the female gonad. Mouse embryonic stem (ES) cells, derived from blastocyst/epiblast stage preimplantation embryos more than 20 years ago (Evans & Kaufman 1981, Martin 1981), have yielded unimaginable progress in identifying the function of many genes within the mammalian genome and in unravelling the mysteries of how the early embryo is formed from a small population of pluripotent cells. The more recent derivation of ES cell lines from the human blastocyst (Thomson *et al.* 1998) has heralded much excitement over potential applications of these cells in understanding human development, providing primary human cell types for drug and toxicology screening (including embryotoxicity), providing a therapeutic product for tissue repair and generating novel *in vitro* models of human disease in the species, cell types and genetic background of interest. The potential to produce gametes as derivatives of mouse and human embryonic stem cells (hESC) (Hubner *et al.* 2003, Clark *et al.* 2004) has also generated much excitement over the utility of these cells as tools in basic developmental research, as well as in more practical applications as alternative sources of gametes for fertility treatments. Moreover, the demonstration that somatic cells, in addition to spermatozoa, can be reprogrammed by the oocyte to yield pluripotent cells in the cloned blastocyst (Wilmut *et al.* 1997) has fuelled the challenge to generate patient-specific stem cells for transplantation, without the need to reprogramme cells within scarce and ethically sensitive human oocytes. The fact that the sources of stem cells described previously have excited our community of reproductive biologists is reflected in the number of their laboratories with a growing interest in gamete or embryo-derived stem cells to inform new directions in this most fundamental area of biology.

Within this focus issue, we are enlightened on the identity of spermatogonial stem cells, on the progress towards derivation of clinical-grade hESC lines, on the different approaches presently used for human ES cell culture, on the differentiation of gametes from ES cells and by a comparison of the strategies employed to reprogramme somatic cells into pluripotent stem cells.

Spermatogonial stem cells provide a unique mammalian system where the entire process of stem cell self-renewal, progenitor formation and all stages of differentiation can be followed in a single organ, *in situ*. Thus, this system has been instrumental in instructing present understanding of the principles of stem cell biology and in defining the role of the stem cell niche in regulating stem cell function. However, the many questions that remain unanswered have been the focus of the Schlatt laboratory, which has revisited recently how spermatogonia expand clonally in non-human primates. In the present issue, Ehmecke and Schlatt (2006) refine this model in light of available data to make predictions of spermatogonial expansion in man. The species-specific kinetics and mechanisms of spermatogonial stem cell expansion in mammals provides yet another example in reproductive biology of the dangers of extrapolating assumptions across species.

Human ES cells have generated as much hype as excitement. While few scientists fail to marvel at the potential of these cells that can be perpetuated in our laboratories and induced to form many parts of the human body, generating a plethora of developmental signals en route, opinions vary as to the reality of their utility in the wide range of suggested applications. Many used to the advantages of inbred mouse ES cell lines for efficient transfection, genetic modification and relative ease of culture/embryoid body formation, find the variability, labour intensiveness and legislative issues surrounding hESC a major barrier to their use as a research tool. At the other extreme, major investments into differentiating hESC into therapeutic stem cell types for rapid advancement to clinical trial are underway. In the interim, a plethora of laboratories are focused in understanding the properties and nuances of the increasing number of hESC lines available worldwide

and in defining how variations in derivation and culture condition can affect their properties. In this issue, Paul De Sousa and colleagues (2006) describe progress towards derivation of hESC lines under the good manufacturing practice (GMP)-type requirements of any product ultimately to be used for transplantation into patients. This goal still represents a major challenge, since hESC have particular requirements for culture on either fibroblast-like feeder cells or on extracellular matrix-type components that are difficult to obtain as defined, purified human products. Until recently, the use of feeder-free cultures required the use of 'conditioned medium' prepared from cultures of human or mouse feeder cells, still representing a non-ideal component for GMP grade cell lines. Several reports of feeder cell-free or conditioned medium-free cultures have provided important alternatives, yet their development and testing in a limited number of hESC lines (often derived from within a single laboratory) requires more generic application in order to prove their utility.

The reasons for difficulty in generic application of culture protocols between hESC lines are unclear, but may well be related to the plethora of conditions used to derive and culture lines (Allegrucci & Young 2006). The review of Skottman and Hovatta (2006) considers the variations in embryo culture protocol, feeder cell type and density, culture matrix, basal culture medium and media additives that, for the most part have not been selected on any empirical, scientific basis. A major barrier to developing *de novo*-optimised media and culture additives is the labour-intensive nature of the culture, with most lines requiring daily medium changes to avoid differentiation and the different protocols required to culture independently derived lines precluding automated culture. Thus, individual laboratories tend to perform small-scale culture on a limited number of independently derived lines, systems not amenable to efficient culture optimisation.

From a reproduction point of view, one of the most exciting derivative cell types from ES cells has been germ cells. Aflatoonian and Moore (2006) review the present status of ES-derived gametes and speculate on the future research required to uncover their developmental competence. Spectacularly, in some cases, evidence of co-differentiation of surrounding gonadal cells has been reported and cells with properties of granulosa cells have also been described from human ES cells. While the speculation that ES-derived gametes may be used for fertility treatments or as recipient oocytes for somatic cell nuclear transfer to facilitate 'therapeutic cloning' has been fuelled by reports of blastocyst formation from *in vitro* maturation of mouse ES-derived oocytes, the developmental competence of gametes conferred by co-evolution of complex ovarian follicle and testis niches should not be underestimated.

Presently, transfer of a somatic cell nucleus into the cytoplasm of a conspecific oocyte is the only method shown to fully reprogramme the mammalian genome and result in the birth of viable and reproductively competent offspring. The limited availability of human oocytes to develop somatic cell nuclear transfer, and the ethical concerns of such a procedure have prompted a range of exciting approaches to reprogramme somatic cells *ex ovo*, or to use interspecific somatic cell nuclear transfer to identify key reprogramming factors. Alberio and colleagues (2006) in this issue review the main approaches and also describe the challenges faced based on our biological understanding of what needs to be reprogrammed within a somatic cell to result in a pluripotent cell phenotype. Once again, many years of research by reproductive biologists in defining how the oocyte reprogrammes and remodels the sperm nucleus has been instrumental in providing the foundation for this novel research. It is no wonder that scientists in the field of reproduction so readily embrace stem cell developments to inform us further on our fascinating quest.

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