Focus on Stem Cells

Germ cells from mouse and human embryonic stem cells

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Abstract

Mammalian gametes are derived from a founder population of primordial germ cells (PGCs) that are determined early in embryogenesis and set aside for unique development. Understanding the mechanisms of PGC determination and differentiation is important for elucidating causes of infertility and how endocrine disrupting chemicals may potentially increase susceptibility to congenital reproductive abnormalities and conditions such as testicular cancer in adulthood (testicular dysgenesis syndrome). Primordial germ cells are closely related to embryonic stem cells (ESCs) and embryonic germ (EG) cells and comparisons between these cell types are providing new information about pluripotency and epigenetic processes. Murine ESCs can differentiate to PGCs, gametes and even blastocysts – recently live mouse pups were born from sperm generated from mESCs. Although investigations are still preliminary, human embryonic stem cells (hESCs) apparently display a similar developmental capacity to generate PGCs and immature gametes. Exactly how such gamete-like cells are generated during stem cell culture remains unclear especially as in vitro conditions are ill-defined. The findings are discussed in relation to the mechanisms of human PGC and gamete development and the biotechnology of hESCs and hEG cells.

Introduction

Detailed investigations of the earliest stages of germ cell development in the human are curbed by the practical and ethical difficulties associated with obtaining human tissue samples. An in vitro model system that can recapitulate the development of human germ cells and gametes would be an extremely valuable research tool, which might lead in due course to novel reproductive toxicological assays and eventually new clinical applications to overcome infertility. Embryonic stem cells (ESCs) are defined as pluripotent stem-cell lines derived from early embryos before the formation of the tissue germ layers (Smith 2006). ESCs are derived usually from the pre-implantation blastocyst and exhibit indefinite proliferative capacity under appropriate conditions in vitro (Evans & Kaufman 1981, Martin 1981, Thomson et al. 1998, Draper et al. 2004). In the last few years, landmark investigations have demonstrated that murine embryonic stem cells (mESCs) can differentiate to primordial germ cell (PGCs) and subsequently early gametes and blastocysts (Hubner et al. 2003, Geijsen et al. 2004). Recently, immature sperm cells derived from mESCs in culture have generated live offspring (Nayernia et al. 2006a, 2006b). Preliminary data indicate that human embryonic stem cells (hESCs) most likely display a similar developmental capacity (Clark et al. 2004, Aflatoonian et al. 2005). This ESC technology offers great potential for new types of reproductive investigations including a readily accessible system to investigate early stages of human gametogenesis including epigenetic modifications of the germline (Allegrucci et al. 2005). Indeed, a range of pluripotent stem cell lines have now been derived from different stages of germ cell development in the mouse and human (Fig. 1). Here, we review the derivation of germ cells and their formation from embryonic stem cells in vitro and discuss how these investigations may provide fresh insight into some of the mechanisms of human germ cell development.

Development of primordial germ cells

Germ cells hold a unique place in the life cycle of an animal as they carry the genome onto the next generation (Donovan & de Miguel 2003, McLaren 2003). In mammals, gametes are derived from a small founder pluripotent population, which segregated early in embryogenesis in the developing yolk sac, become PGCs. Elucidating the mechanisms controlling human PGC and gamete development is crucial for...
understanding the aetiology of various aspects of infertility. For example, it has been hypothesised that endocrine disrupting chemicals either in the environment or the workplace may interfere with early germ cell development thereby increasing susceptibility to testicular cancer and infertility in adulthood – the so-called ‘testicular dysgenesis syndrome’ (Skakkebaek et al. 2001). Indeed, there is growing evidence for effects of environmental chemicals on the various early stages of germ cell and gamete development (Baillie et al. 2003). Recently, organochlorines were implicated in affecting the ratio of X and Y sperm in human semen (Tiido et al. 2005) and transient exposure of pregnant rats in early gestation to the fungicides and endocrine disrupting chemicals vinclozolin (antiandrogenic compound) or methoxychlor (an oestrogenic compound) increased the incidence of male infertility in offspring (Anway et al. 2005). Significantly, this infertility was transferred through the male germ line to subsequent generations possibly indicating modification to epigenetic pathways.

Specification of PGCs, their proliferation, maintenance, then differentiation to primary oocytes and spermatogonia (precursor spermatogonia stem cells) all have a profound bearing on the number and function of germ cells that are available subsequently for gametogenesis. Over many years, the activity of tissue non-specific alkaline phosphatase (TNAP) has been used to mark PGCs and monitor their transition from the base of the allantois, through the hindgut to the dorsal body wall where they enter into the genital ridges of the gonadal anlagen (McLaren 2003). The high expression of this enzyme in PGCs is a feature also shared with ESCs, embryonic germ (EG) cells and embryonal carcinoma (EC) cells, all of which have pluripotent capabilities. PGC migration (whether active or passive) occurs at about 7–10 days post-conception (dpc) in the mouse and between weeks 5 and 8 of human gestation (Freeman 2003, Molyneaux & Wylie 2005). Because of the difficulties of undertaking detailed investigations of early human fetal development, relatively little is known of the specification of human PGCs, although it is probable that common signalling pathways occur in mammals and possibly all vertebrates (Donovan & de Miguel 2003). In the mouse, PGCs arise from the proximal epiblast, a region of the early mammalian embryo that also contributes to the first blood lineages of the embryonic yolk sac (Ginsburg et al. 1990). Recent studies indicate that as early as 6.25 d.p.c, germline competence can be identified in a founder population of perhaps as few as six epiblast cells that express the
protein Blimp1 (B-lymphocyte-induced maturation protein 1, McLaren & Lawson 2005, Ohinata et al. 2005, Vincent et al. 2005). Blimp1 was first identified as a transcriptional repressor that enables the further differentiation of immunoglobulin-secreting plasma cells by inhibiting the expression of genes involved in alternative B-cell development. Mutant null-allele mice lacking Blimp1 generate very few PGCs and those that develop lack normal migratory behaviour, unlike the cells of wild-type individuals where PGCs will multiply rapidly as they migrate to the genital ridges to eventually become non-migratory gonocytes (Vincent et al. 2005).

In females, the gonocyte surrounded by a cortical interstitial layer initiates meiosis and becomes a primary oocyte and follicle, thereby ending precursor proliferative potential. In males, the gonocyte surrounded by the fetal sex cord of the gonadal ridge (pre-seminiferous tubules) arrests in G0/G1 of mitosis as a prospermatogonium, but retains a proliferative precursor potential. Following birth, prospermagonia migrate to the basement membrane of the seminiferous tubule and differentiate into spermatogonial stem cells (SSCs). Like adult stem cells, SSCs can both self-renew and provide daughter cells, which differentiate into one or more terminal cell types (Brinster 2002).

Germ cell competence is induced in the murine proximal epiblast in response to signals emanating from the extra embryonic ectoderm including the synergistic action of the growth factors, particularly bone morphogenetic proteins (BMP) 4 and 8b; both members of the transforming growth factor-β (TGF-β) superfamily of secreted proteins (Shimasaki et al. 2004). Mature BMP4 is a dimer that binds to and signals through heteromeric receptor complexes and downstream SMAD proteins. However, BMP4 or BMP8b alone are unable to induce PGCs from cultured epiblast, while they can when combined, which suggests that signalling for various BMPs may occur through separate receptor complexes. BMP2, a close relative of BMP4, is expressed in visceral endoderm at the time of PGC specification, and inactivation of BMP2 results in a reduction in PGC number, revealing a function of visceral endoderm in PGC generation in the mouse at least (Lawson et al. 1999, Ying et al. 2001, de Sousa Lopes et al. 2004, Shimasaki et al. 2004).

The genes fragilis and stella also have key roles in germ cell competency and development. Fragilis is a transmembrane protein and part of a larger interferon-inducible family of genes that is evolutionarily conserved and has human homologues. Interferon-inducible proteins such as fragilis have an anti-proliferative function and may serve to increase the length of the cell cycle in PGCs. As germ cell fate is induced, there is only transient expression of fragilis, but this gene is also expressed in ESCs and embryonic germ (EG) cells, suggesting a potential role in pluripotency status (Saitou et al. 2002). Similarly, stella may have a function during the development of pluripotency and is associated with chromatin remodelling or RNA processing. It is expressed in the oocyte, through pre-implantation embryo development and in germ cell tumours (Payer et al. 2003). Stella-positive nascent germ cells exhibit repression of homeobox genes, which may explain their escape from a somatic cell fate and the retention of pluripotency (Saitou et al. 2002). Transgenic mice have been generated that express a green fluorescent protein (GFP) – stella reporter transgene, which appears to accurately mark PGC development (Payer et al. 2006).

A number of other factors have been implicated in PGC derivation and maintenance. Immunohistochemical analyses demonstrate that mouse vasa homologue (mvh) protein was exclusively expressed in PGCs just after their colonisation of embryonic gonads and in germ cells undergoing gametogenic processes until the post-meiotic stage in both males and females (Toyooka et al. 2000). The tyrosine-kinase receptor c-kit and its ligand, stem cell factor (SCF), are also essential for the maintenance of PGCs in both sexes. In the adult testis, the c-kit receptor is re-expressed in differentiating spermatogonia, but not in spermatogonial stem cells, whereas SCF is expressed by Sertoli cells under follicle-stimulating hormone stimulation (Rossi et al. 2000).

Another set of genes involved in germ cell development is DAZ (deleted in azoospermia) genes. Men with deletions encompassing the Y-chromosome DAZ genes have few or no germ cells, indicating they are defective in the formation or maintenance of germ cells. A DAZ homolog, DAZL (DAZ-Like), found in diverse organisms, including humans is required for germ cell development in males and females. Significantly, PUM2, a human homologue of pumilio, a protein required to maintain germ line stem cells in Drosophila and Caenorhabditis elegans, forms a stable complex with DAZ through the same functional domain required for RNA binding, protein–protein interactions (Moore et al. 2003) suggesting mechanisms of germ cell development are highly conserved.

The POU domain transcription factor Oct4 has been shown to have a role in PGC survival. It has been known for some time that this transcription factor is crucial for maintaining pluripotency in the inner cell mass of the blastocyst and in ESCs. By use of conditional gene targeting with the Cre/LoxP system, Kehler et al. (2004) showed that loss of Oct4 leads to apoptosis (Kehler et al. 2004). In human fetal tissue, Oct4 is strongly expressed in migrating PGCs as well as in human germ cell tumours and EG cells (Looijenga et al. 2003, Rajpert-De Meyts et al. 2004). Oct4 expression is down-regulated rapidly in the human female gonad and silenced as oocytes enter the first meiotic prophase. In contrast, the same process occurs much more gradually in the male with Oct4 expression often persisting in some gonocytes and infantile spermatogonia. This observation has prompted the suggestion that differential germ cell expression of

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Oct4 between the sexes might contribute to the fact that germ-cell derived cancer is much higher in men than women (Rajpert-De Meyts et al. 2003).

Thus, some of the genes that play a crucial role in germ cell differentiation are Blimp1, Stella, Fragilis, c-Kit, vasa and DAZL. Their expression is stage-specific, therefore, allowing solid identification of germ cells at different developmental phases. In addition to the expression of these genes, other markers associated with germ cell development are non-specific alkaline phosphatase activity, the stage-specific embryonic antigen 1, the transcription factor Oct4 and beta1- and alpha6-integrins (Lacham-Kaplan 2004; Fig. 2).

**Embryonic stem cells to germ cells**

ESCs have been defined as pluripotent (Smith 2006) as they differentiate along somatic cell lineages but were not believed to form either trophoblast or germline cells. However, it may be more appropriate to use the term totipotent for hESCs as they can spontaneously generate trophoblast and trophoblast-stem cells, while mESCs form trophoblast if Oct4 expression is conditionally down-regulated (Xu et al. 2002, Hay et al. 2004, Harun et al. 2006). Moreover, ESCs can apparently form germ cells which have a totipotent potential. In the last few years it has become clear that murine ESCs can develop into PGCs in vitro (Hubner et al. 2003, Geijsen et al. 2004, Payer et al. 2006) and after further culture may occasionally form early spermatids or oogonia, which form follicle or ovarian-like structures and oocytes (i.e. having morphological similarities to follicles and gametes from gonads) that subsequently develop to blastocyst-like structures, presumably due to parthenogenetic activation (Hubner et al. 2003, Geijsen et al. 2004, Lacham-Kaplan et al. 2006). Clearly, as these germ-cell derivatives have not arisen from true gonadal structures it is crucial that biological function is properly characterised. Some biological functions of mESC-derived spermatids were tested in vitro using ESCs initially carrying an Oct 4 promoter-driven GFP reporter gene, which enabled the tracking of these cells (Hubner et al. 2003, Geijsen et al. 2004). When haploid spermatids generated from these GFP–ESCs were isolated by flow cytometry and injected intracytoplasmically into oocytes, the diploid chromosome complement was restored and green fluorescent blastocysts generated. Noce and co-workers (Toyooka et al. 2003) showed that Mvh-LacZ murine germ cells (transgene with germ cell- specific mouse vasa homolog and LacZ reporter) generated in vitro from mESCs could also develop to some extent in vivo. To achieve this, cells were aggregated with day 12.5–13.5 (d.p.c) gonadal tissue, which formed seminiferous tubules (but separate from host tubules) within 6–8 weeks when co-transplanted under the testis capsule. These tubules participated in spermatogenesis to produce elongated β-gal-stained spermatids. This demonstrated that such mvh-lacZ ESCs formed PGCs with developmental potential to integrate into a somatic epithelium and undergo meiosis to form early sperm cells. There had remained considerable uncertainty as to the biological fidelity of these various culture systems, but Nayernia et al. (2006a, 2006b) recently produced viable transgenic offspring from sperm produced from mESCs using a novel two-stage culture system. First, mESCs were transfected with the reporter gene Stra8-EGFP, a retinoic acid (RA)-responsive gene expressed in pre-meiotic mouse germ cells and fused with enhanced green fluorescent protein gene construct. Following ESC culture, green cells expressing the gene and therefore presumably marking pre-meiotic germ cells were isolated by fluorescence-activated flow cytometry and cultured initially in the presence of RA as a proliferative cell line. Subsequently, the cells were transplanted with a second fusion reporter gene Prm1-dsred (protamine1 gene fused to red fluorescent protein gene construct) to identity post-meiotic sperm cells. After further RA induction for a few days, red post-meiotic sperm cells were isolated and microinjected

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**Figure 2** Schematic diagram of the developmental stages of mammalian germ cell development and some of the factors and genes (italics) involved. The genes have been used as markers of PGC and gamete development from ESCs in culture.
into mouse eggs to generate viable offspring although these exhibited abnormalities and died prematurely (5 days to 5 months).

While investigations with hESCs are more preliminary, they also show that spontaneous or induced differentiation in culture can generate cells displaying mRNA expression profiles and cell surface markers consistent with PGCs (Clark et al. 2004, Clark & Reijo Pera 2006) and of subsequent meiosis. In our lab, PGCs (and on rare occasions, early spermatid cells but so far not oocytes) have been identified after the appropriate culture conditions (Aflatoonian et al. 2005).

Gamete determination

During murine and human ESC culture, markers of female germ cells are expressed in both XX and XY cell lines (Toyooka et al. 2003, Geijsen et al. 2004). The prevailing hypothesis to date has been that whatever their complement of sex chromosomes germ cells are cell – autonomous and intrinsically programmed to undergo meiosis, enter prophase and develop as oocytes, unless otherwise prevented from doing so by a meioisis inhibiting factor(s) (McLaren 2003). In this case, it would be unsurprising for both male and female ESC lines to display female germ cell markers, since culture conditions may be sub-optimal and lack meiosis inhibition. However, recent studies (Bowles et al. 2006, Koubova et al. 2006) indicate that instead of an intrinsic programme to enter meiosis, germ cells respond to the external signal of RA and its metabolism. Thus, in the embryonic mouse ovary, RA induces germ cells to express the pre-meiotic marker Stra-8 (stimulated by retinoic acid 8) and initiate meiosis. By contrast, in the embryonic mouse testis, RA is metabolised and inactivated by P450 enzyme CYP26 (B1) thereby preventing early germ cell entry into meiosis with down-regulation of genes such as SCP3 (synaptonemal complex protein; associated with meiotic events) and the induction into the alternative pathway of mitotic arrest as GO/G1 prospermatogonia. Therefore, the induction of presumptive PGCs into meiosis in culture medium containing RA might be expected although local concentrations within cell aggregates may differ significantly and affect the timing. When RA has not been added as a supplement to basal medium, the spontaneous induction of PGCs from ESCs might potentially still arise if RA is endogenously generated by ESC derivatives. Concentrations of RA in plasma or serum (or serum replacement) that supplement ESC culture medium are normally in the 2–8 nM range (Eckhoff & Nau 1990), much lower than the 1–5 μM concentrations often used with induction protocols. However, the sensitivity of cells to RA can vary considerably depending on composition of medium.

When hESCs are cultured the process of sex determination seems even more dysregulated as markers of both male and female germ cell development have been detected regardless of the sex of the cell line (Clark et al. 2004, Aflatoonian et al. 2005). The implication from these findings is that RA or possibly other factors affecting meiosis and gamete determination are present in the culture conditions possibly generated by male and female hESCs. For example, for male germ cell development in vivo, seminiferous epithelium with Sertoli cells is required. This is normally dependent on the expression of the transcription factor SRY, the gene of which is normally located on the Y chromosome. Therefore, for XX hESCs to generate presumptive male germ cells either an artefactual differentiation of Sertoli cells occurs, for example, by female supporting cells differentiating to a Sertoli-like phenotype (Adams & McLaren 2002) or meiosis inhibitor factors are produced (or metabolised in the case of RA) from a different origin. Interestingly, in vitamin A-deficient adult mice, where only spermatogonia and Sertoli cells populate the seminiferous epithelium, the addition of retinoids such as RA and retinol may induce the resumption of spermatogenesis (Baleato et al. 2005).

The germ cell niche and ESCs

An intriguing aspect of the generation of ESC-derived germ cells in vitro is how the normal checkpoints of the developmental process, which would normally span a timeline from early fetal development to puberty and lead to arrest of cells at different stages, seem to be overcome and then apparently compressed into a relatively much short culture period. It has been suggested that in the absence of environmental cues, germ cells may develop according to an intrinsic clock. Using stella–GFP murine ESCs, Payer et al. (2006) showed somewhat surprisingly that ESC colonies displayed a well-defined subpopulation of Stella-positive cells and unlike the uniform distribution of cells expressing Oct4 or TNAP. Significantly, there was overlap of expression of Oct4 and stella in some cells and, since this combination of markers identifies germ cells it may indicate a population of germ cell-like ESCs (Payer et al. 2006). Whether stella-positive ESCs are destined to form PGCs in the mouse and whether their specification requires subsequent exposure to a set of environmental cues, awaits further investigation but these results suggest that there is perhaps an inherent propensity of ESC culture systems to form PGCs. Thereafter, these cells may create a stem cell niche continuum for induction of germ cell development.

Exactly how ESC cultures may mimic the somatic environment that encapsulates either developing oogonia (the follicle) or the sperm stem cell (semiferous epithelium) is unclear. The appropriate growth factor and hormonal microenvironment required to support and sustain these complex niches probably depends to some extent on the type of culture system adopted for the
Monolayer adherent cultures of ESCs can be allowed to differentiate directly to form an appropriate niche or ESCs can be induced to aggregate to form embryoid bodies that form a more three-dimensional micro-environment. In conjunction with these two culture methods, ESCs can be initially co-cultured with feeder layers (i.e. mouse embryonic fibroblasts and neonatal gonadal cell culture) or conditioned-medium from feeder cells and various growth factor and serum supplementation. Generally, these culture conditions attempt to create an environment conducive for germ-cell proliferation and differentiation but these conditions are far from being specific. Apart from the absence of a defined culture medium, the various procedures for passaging ESCs (i.e. enzymatically with trypsin to single cells or as clumps with collagenase or manually), the influence of feeder cells and extracellular matrix (e.g. Matrigel) and the cell line itself can all contribute to whether germ cells are specified. If a progenitor cell population is not selected (i.e. using fluorescent reporter systems), then only a small proportion of cells (usually less than 5%) will normally display PGC or gonocyte/spermatogonia markers, but the fact that some cells progress further to a post-meiotic stage seems remarkable given the complex hormonal requirements of gamete development. Interestingly, oestradiol production (50–100 pg/ml) was detected after 12 days in mESC cultures that generated follicle-like structures (Hubner et al. 2003), while appreciable levels (60 pg/ml) of dihydrotestosterone can be detected in hESCs cultures that generate PGCs and spermatids (Aflatoonian & Moore, unpublished). Presently, the source of steroidogenesis is unclear. It may originate from additives to the culture medium (i.e. serum or serum replacement supplement) or gonadal cell types (granulosa, Leydig cells) that co-differentiate with germ cells. This appeared to be the case for oocytes generated from mESCs where increased oestradiol levels were correlated with increased proportion of apparent follicular cells (Hubner et al. 2003). Alternatively, steroids may be produced from non-reproductive cell phenotypes (e.g. liver or adrenal cells) that could conceivably differentiate from ESCs in culture at the same time as germ cells.

The proliferative capacity of germ cells in culture will depend to some extent on their fate. Since, at least in vivo germ cells are programmed by default to enter meiosis, the efficiency at which ESC cultures will generate germ cells will depend on the initial rate of differentiation to PGCs and subsequently whether factors are present to divert development down spermatogenesis when mitotic proliferation of prospermatogonia can potentially occur. Recent studies (Hamra et al. 2005, Ryu et al. 2005) indicate that the self-renewal of these spermatogonial stem cells (SSCs) in rodents (and possibly all mammals) is dependent on glial cell line-derived neurotrophin factor (GDNF), the GDNF-family receptor α-1 and basic fibroblast growth factor, providing candidate factors to be tested in ESC systems.

In the mouse, pluripotency of ESCs is mirrored also by EG cells (Matsui et al. 1992, Resnick et al. 1992) but additionally extends to a population of SSCs in the neonatal mouse testis (Kanatsu-Shinohara et al. 2004) and perhaps more remarkably in adult mouse testis (Guan et al. 2006). These adult SSCs can be transformed in culture to ES-like cell lines displaying pluripotency both in vitro and significantly in vivo. When injected into mouse pre-implantation blastocysts they contribute to the development of all the major organs and display germline transmission (Guan et al. 2006) and therefore are termed multipotent adult germine stem cells. The obvious conservation in many of the mechanisms for germ cell development in mammals suggests that it might be possible to develop similar cell lines from human adult spermatogonial populations although the translation from the murine to the human system may not be straightforward. While human EG cell lines have been reported (Shamblott et al. 1998), their proliferative and pluripotent capacity presently as true EG cells has been questioned (Turnpenny et al. 2003, Aflatoonian & Moore 2005).

Since the evidence is becoming more compelling that in culture ESCs can generate PGCs and germ cells, an important question (and the subject of considerable debate) is whether germ cells can be derived (or transdifferentiated) from adult stem cells residing outside the gonad? In the mouse, it has been claimed that germ cells can be derived from bone marrow (BM) and peripheral blood cells (Johnson et al. 2004, 2005, Nayernia et al. 2006b) and BM-derived germ cells can repopulate the ovarian follicular reserve (Johnson et al. 2004, 2005). However, the latter investigations were criticised (Telfer et al. 2005) and subsequently have not been substantiated (Eggan et al. 2006). Germ-cell derivation from pig fetal skin cells in vitro has also been reported (Dyce et al. 2006) with the formation of morphological structures resembling follicles, that secrete oestradiol and progesterone; and oocyte-like cells displaying markers of germ cells (oct4, vasa, GDF9b) or exhibiting the meiosis marker, SCP3 and displaying apparent parthenogenetic embryo structures. Clearly, if any of these recent reports can be fully corroborated, they may indicate that PGC and germ cell formation in vitro and possibly in vivo is much more plastic than previously believed (Fig. 3). At present, it is very difficult to reconcile some of these observations with known reproductive physiology in mammals, and an alternative explanation is that detection of many germ cell markers is due to aberrant expression or detection in culture and not due to true germ cell phenotype. The use of a wider battery of specific protein and functional phenotypic assays are required to resolve this issue.
Conclusion

ESCs differentiation remains hard to control and accurately monitor in vitro but with the advent of highly specific transgene reporter systems to monitor germ-cell differentiation, the evidence that primordial germ cells can be derived readily from mouse ESCs in vitro appears strong. Moreover, there are growing data indicating that very similar processes are likely to occur with hESCs. It seems that occasionally gametes, albeit immature, can be generated under appropriate culture conditions although their complete developmental competence remains uncertain. The promiscuous nature of spontaneous ESC differentiation in culture makes it especially difficult to determine at present whether PGC and germ cell development follow similar programmes to those occurring in vivo or whether the conditions merely provide suitable conducive conditions that permit an intrinsic developmental process but not necessarily the same as the gonadal environment. For example, the findings that the development of both haemopoietic plasma cells and PGCs depend on expression of the same protein (blimp1) indicate conservation in these differentiation systems and therefore there is potential for interference between pathways during in vitro development. However, with careful selection of cells with the appropriate markers, it should be feasible to devise stage-specific protocols (with specific growth factors) for differentiation of ESCs to PGCs and subsequently PGCs to gonocytes and male or female germ cell fate. Given the continuing advances in the proliferation and maturation of germ cells in vitro, the production of in vitro gametes may be a practical proposition in the not too distant future. However, there remains major uncertainty about the genetic/epigenetic processing of germ cells in vitro and very careful consideration would need to be given to safety aspects of such cells if ever they are to be used for clinical applications.

Acknowledgements

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 5 May 2006
First decision 7 August 2006
Accepted 1 September 2006