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Stem cells for reproductive medicine

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ABSTRACT

The generation of various pluripotent stem cell lines provides a new route to investigate developmental process of germ cell and embryo development, which until now was difficult to access in the human. In the future these cells may be used for new therapies in reproductive medicine. This brief review outlines the development of germ cells and their pluripotent capabilities, how embryonic and germline stem cells can mimic developmental processes *in vitro* and generate gamete and trophoblast phenotypes for research and potential treatments.

1. Introduction

The last 30 years has seen major advances in the treatment of infertility with the introduction of such treatments as hormonal induction of follicular development and ovulation; *in vitro* fertilisation and embryo transfer; intracytoplasmic sperm injection; and embryo and egg cryopreservation. For many patients, a combination of these assisted conception procedures can bypass critical physiological barriers such as spontaneous ovulation, fertilisation and embryo transport to alleviate infertility. However, when there is a primary failure of germ cell production as a result of developmental abnormalities, age, disease or toxic insult (e.g. chemotherapy), or embryo implantation failure, the clinician is often severely hampered in providing an effective treatment. In the last few years, many investigations have moved to understanding the basis of early germ cell development with the hope that in time, treatments can be developed that stimulate *de novo* gamete development from the primary stem cells to rescue the gonad. Moreover, a new paradigm for studying the very earliest stages of germ cell commitment and differentiation, or early stages of trophoblast and placental differentiation has been created by the finding that embryonic stem (ES) cells can differentiate to a germ cell lineage and early gametes, and to trophoblast lineages in culture. It is anticipated that this research will provide a more accessible route to investigate some of the origins of human gonadal dysfunction (e.g. testicular cancer, early menopause), the effects of environmental chemicals on germ cells and embryos (Skakkebaek et al., 2001; Anway et al., 2005) and the developmental processes of early placentation. It also raises prospects of novel treatments for infertility sometime in the future.

The relationship between germ cells and embryos *in situ* and pluripotent stem cells *in vitro* has become increasingly complex as germ cell lines at different stages of development are demonstrated to have pluripotent/multipotent potential and show germ cell differentiation (Fig. 1).

2. The primordial germ cell

The primordial germ cell (PGC) is the primary undifferentiated stem cell of spermatozoa and oocytes. Since PGCs provide the fundamental hereditary link from one generation to another, they are in this respect both immortal and totipotent. Early in evolution, unicellular organisms segregated immortal germplasm from the mortal soma probably as a way of protecting the fidelity of the genome when nutrients are scarce. This evolutionary legacy is still manifest in mammals today in the early foetal segregation of PGCs in extra embryonic tissue (Donovan and de Miguel, 2003; McLaren, 2003), which subsequently migrate to the gonadal anlagen and differentiate to the gonocytes; progenitor cells committed to form oocytes or spermatozoa depending on the genetic sex of the individual. In the female, the gonocyte surrounded by a cortical interstitial layer initiates meiosis and becomes a primary oocyte and follicle, thereafter ending precursor proliferative potential. In the male, the gonocyte surrounded by the foetal sex cord of the gonadal ridge (pre-seminiferous tubules) arrests in G0/G1 of mitosis as a prospermatogonium but maintains a proliferative precursor

Abbreviations: BM, bone marrow; CTBS, cytotrophoblast stem; ES, embryonic stem; mES, mouse embryonic stem; hES, human embryonic stem; EG, embryonic germ; GSC, germline stem cell; maGSC, mouse adult germline stem cell; PGC, primordial germ cell; SSC, spermatogonial stem cell.

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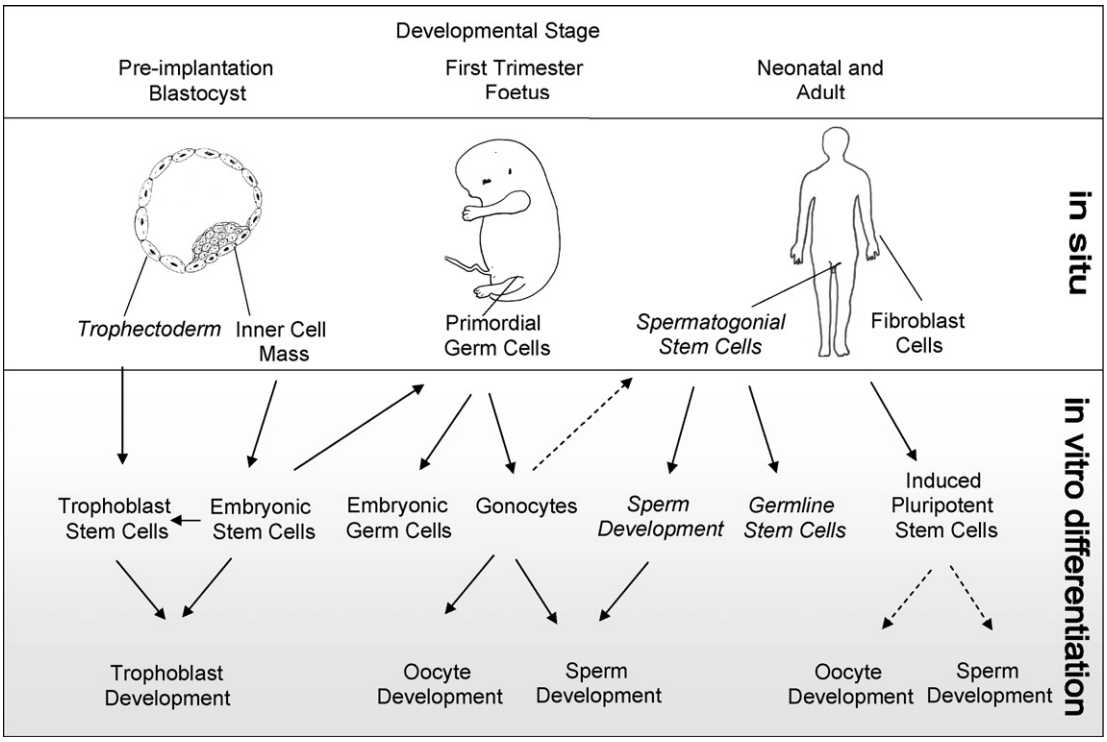

Fig. 1. The complex inter-relationship *in vitro* between different pluripotent stem cells and embryo and germ cell development (dotted arrows indicates potential differentiation) which has evolved over the last few years. Bold: from human and mouse; italics: from mouse.

potential. Following birth, prospematogonia migrate to the basement membrane of the seminiferous tubule and differentiate into spermatogonial stem cells (SSCs) with self-renewal capacity. Therefore, PGCs have a relatively short window of proliferative activity although they can be transformed *in vitro* to embryonic germ cells with prolonged proliferative and pluripotent capacity (Fig. 1) as is also the case for some of the later stages of germline stem cells.

While ethical constraints limit our knowledge of the specification of human PGCs, it is clear that common signalling pathways operate across mammals and possibly all vertebrates (Donovan and de Miguel, 2003). In the mouse, PGCs originate in the proximal epiblast and by 6.25 days post conception (d.p.c.) a small germline founder population can be identified that express the protein Blimp1 (B-lymphocyte-induced maturation protein 1, Ohinata et al., 2005; Vincent et al., 2005; McLaren and Lawson, 2005). Blimp1 was initially identified as a transcriptional repressor that enables the further differentiation of immunoglobulin-secreting plasma cell by inhibiting the expression of genes involved in alternative B-cell development. Mutant null-allele mice lacking *Blimp1* generate very few PGCs, and any that do still develop lack the normal migratory behaviour towards the genital ridge (Vincent et al., 2005). Germ cell competence is induced in response to signals secreted by extra embryonic ectoderm including the synergistic action of the growth factors; particularly bone morphogenic proteins (BMP). In their mature dimeric form BMPs bind and then signal through heteromeric receptor complexes and downstream SMAD proteins (Shimasaki et al., 2004). Induction of PGCs seems to require BMP4 or BMP8b in combination possibly indicating that signalling for various BMPs occur through separate receptor complexes.

The genes *fragilis* and *stella* also appear to have major roles in germ cell competency and development. Fragilis is a transmembrane protein and a member of a large interferon-inducible family of genes that is evolutionarily conserved with human homologues. A characteristic of interferon-inducible proteins is their anti-proliferative function and fragilis may serve to increase the length of the cell cycle in PGCs. During induction of germ cell fate, there is only a transient expression of *fragilis* but this gene is also expressed in ES cells and embryonic germ (EG) cells indicative of a potential role in pluripotency status (Saitou et al., 2002). Likewise, the gene *stella* may have a function during the development of pluripotency. It is expressed in the oocyte, through pre-implantation embryo development and in germ cell tumours (Payer et al., 2003). *Stella*-positive germ cells exhibit a repression of homeobox genes, which is probably important in order to escape a somatic cell fate (Saitou et al., 2002). Transgenic mice expressing a green fluorescent protein (GFP)—*stella* reporter transgene can be utilised to accurately follow PGC development (Payer et al., 2006).

A number of other factors have been implicated in PGC derivation and maintenance (Fig. 2). The tyrosine-kinase receptor c-kit and its ligand, stem cell factor (SCF), are 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 FSH stimulation (Rossi et al., 2000). Also the POU domain transcription factor Oct4 has been shown to have a role in PGC survival. This transcription factor is a 'gatekeeper' for maintaining pluripotency in the inner cell mass cells of the blastocyst and in ES cells. By use of conditional gene targeting with the Cre/LoxP system, Kehler et al. (2004) showed that loss of Oct4 leads to apoptosis. In keeping with their pluripotent capability, Oct4 is strongly expressed in migrating PGCs in human foetal tissue as well as in human germ cell tumours and EG cells (Looijenga et al., 2003; Rajpert-De Meyts et al., 2004). On the other hand, Oct4 expression is down regulated rapidly in the human female gonad and silenced as oocytes enter the first meiotic prophase. In contrast, the equivalent process occurs much more gradually in the male