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Review – Mini-Series on Germ Cell Development

# On the formation of germ cells: The good, the bad and the ugly

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ABSTRACT

Mammalian germ cells are powerful cells, the only ones that transmit information to the next generation ensuring the continuation of the species. But "with great power, comes great responsibility", meaning that germ cells are only a few steps away from turning carcinogenic. Despite recent advances little is known about germ cell formation in mammals, predominantly because of the inaccessibility of these cells. Moreover, it is difficult to pin down what in essence is characteristic of a germ cell, as germ cells keep changing place, morphology, expression markers and epigenetic identity. Formation of (primordial) germ cells in primate ES cell cultures would therefore be helpful to identify molecular signalling pathways associated with germ cell differentiation and to study epigenetic changes in germ cells. In addition, the *in vitro* derivation of functional germ cells from ES cells could be used in combination with therapeutic cloning to generate patient-specific ES cell lines, and can have applications in animal breeding. In this review we present the state-of-the-art on how mouse and human germ cells are formed *in vivo* (the good), we discuss the link between germ cells, pluripotency and germ cell tumours (the bad) and show that despite continuous progress in trying to differentiate germ cells *in vitro* (the ugly) the generation of functional germ cells is still a real challenge.



**Contents**



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## 1. What defines a *bona fide* germ cell?

### 1.1. Germ cell expression markers

One of the most striking features of mammalian germ cells is their dynamic character. These special cells manage to be ever changing without loosing their underlying pluripotency potential that manifests itself every time fertilization occurs, completing the germ cell life cycle (Fig. 1). Remarkable, despite their pluripotency qualities, germ cells shift their location in the organism and keep changing their morphology, the character of their organelles, their transcriptional profile and epigenetic signature.

In organisms that show a "preformation" or "inheritance" mode of germ cell formation, germ cells are easily distinguished throughout the life cycle by the presence of "germ plasm", a cell-dense association of mRNAs and mRNA-binding proteins such as VASA, DAZL and MILI homologues. However, in mammals and in particular in mice, which show an "inductive" or "regulative" mode of germ cell formation, there seems to be no "germ plasm" components localized in the early embryo from fertilization until the onset of gastrulation. Whether or not "germ plasm" components are present during the early embryonic development of other placental mammals, monotremes or marsupials still remains to be determined.

Throughout the lifetime of a mammalian germ cell, from the time the germ cells are specified, which in the mouse occurs at embryonic day (E)7.25, until the completion of gamete maturation, not a single germ cell-specific marker has been identified to be continuously expressed. This lack in continuity of expression of specific germ cell markers has greatly hampered our understanding of mammalian germ cell development. In particular, there is a radical change in the expression of pluripotency makers (including NANOG, SOX2 and OCT4), which are present in the primordial germ cells before they arrive in the gonads, but are dowregulated at slightly different timing in the germ cells after they arrive in the gonads, stop proliferation and undergo sex differentiation (Pesce et al., 1998; Avilion et al., 2003; Yamaguchi et al., 2005; Payer et al., 2006; Maldonado-Saldivia et al., 2007; Western et al., 2005). On the other hand, the expression of germ cell markers characteristic of "preformed" germ cells (including VASA, DAZL and MILI homologues) becomes detectable in germ cells upon arrival in the gonads, cell cycle exit and sex differentiation (Toyooka et al., 2000; Ruggiu et al., 1997; Seligman and Page, 1998; Kuramochi-Miyagawa et al., 2001). VASA, DAZL and MILI homologues are involved in germ cell-specific mRNA localization and processing and in mammals this seems to become important in germ cells after their arrival and settlement in the gonads.

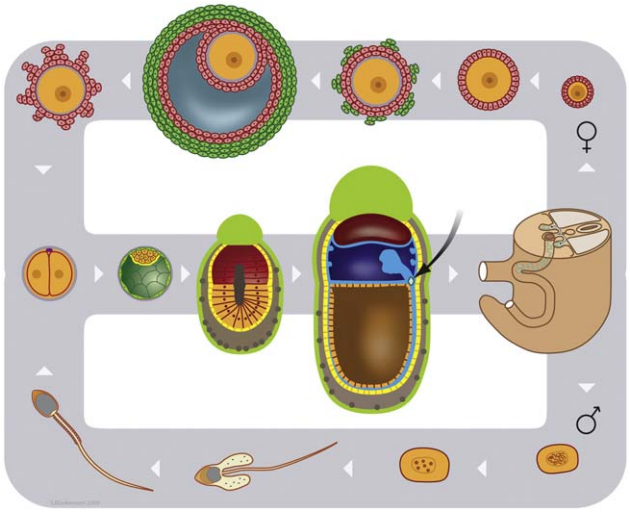


**Fig. 1.** The life cycle of murine germ cells. After fertilization the zygote starts cleaving forming a 2-cell embryo that continues cleaving giving rise to the blastocyst (middle row). The blastocyst consists of the trophectoderm (green), the primitive endoderm (yellow) and the inner cell mass (orange). After implantation, the inner cell mass/epiblast (orange) undergoes cavitation and epithelializes. During gastrulation, a new germ layer is formed, the mesoderm (blue). The primordial germ cells (PGCs) are specified at E7.25 (black arrow) at the base of the mesodermal allantois. Thereafter, the PGCs migrate through the forming hindgut and the dorsal mesenterium to finally lodge in the genital ridges. Upon sex determination, female (oogonia) and male (spermatogonia) germ cells follow different fates. In the ovary (top row), the oocytes mature in their follicles and are then realised during ovulation. In the testis (bottom row), spermatogenesis takes place to generate motile sperm cells able to fertilize a mature oocyte.

### 1.2. Germ cell behaviour and morphology

Mammalian germ cells are per definition very susceptible to environmental cues, in particular because they have to migrate from their location of origin, at the base of the allantois to the future genital ridges (Fig. 1). They therefore have to identify and respond to specific positional clues during the migratory period (E7.5–E10.5). Germ cells show extensive pseudopodia during migration though the hindgut (regulated by the KITL-KIT signalling pathway) and during the migration up the dorsal mesenterium (regulated by the SDF1-CXCR4 signalling pathway) (Ara et al., 2003; Molyneaux et al., 2003; Gomperts et al., 1994; Gu et al., 2009; Mintz and Russell, 1957; Buehr et al., 1993; Stebler et al., 2004). The dynamic expression of extracellular molecules and extracellular matrix binding proteins (including integrins) in the germ cells also plays an important role during migration (De Felici et al., 2005; Cachaco et al., 2003; Chuva de Sousa Lopes et al., 2005; Kunwar et al., 2006).

During their life cycle, the germ cells exhibit a continuous contortion of the cell cycle, including periods with lengthy mitosis (Tam and Snow, 1981), synchronous mitosis with incomplete cytokinesis in germ cells entering the gonads (Pepling and Spradling, 1998, 2001), followed by meiosis in female germ cells and cell cycle arrest in male germ cells, a consequence of sex determination. Later in life, male germ cells reacquire mitotic potential to form a true stem cell population (spermatogonia A) that either self-renews or undergoes differentiation that includes both mitotic rounds and meiosis to form sperm. Female germ cells on the other hand exhibit an extreme form of asymmetric division during meiosis to extrude the first and second polar bodies.

### 1.3. Germ cell epigenetic signature

Epigenetically, the germ cells are also ephemeral. In the mouse, between germ cell specification at E7.25 and sex differentiation at E12.5, the germ cells will gradually exchange histone modifications and histone variants (Seki et al., 2005; Hajkova et al., 2008), reactivate the silent X chromosome in females (Chuva de Sousa Lopes et al., 2008; Sugimoto and Abe, 2007), remove parental imprints (Hajkova et al., 2002; Sato et al., 2003) and undergo DNA demethylation (Seki et al., 2005). Primordial germ cells (PGCs) during the period of migration and proliferation do not truly self-renew, but instead seem to adopt a slightly different "epigenetic" flavour with every cell division.

## 2. The good: germ cell formation *in vivo*

### 2.1. When are germ cells formed in the mouse?

Even though it is difficult to pinpoint a general characteristic for germ cells during their life cycle, it is clear that germ cells are