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Modeling human infertility with pluripotent stem cells

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ABSTRACT

Human fertility is dependent upon the correct establishment and differentiation of the germline. This is because no other cell type in the body is capable of passing a genome and epigenome from parent to child. Terminally differentiated germline cells in the adult testis and ovary are called gametes. However, the initial specification of germline cells occurs in the embryo around the time of gastrulation. Most of our knowledge regarding the cell and molecular events that govern human germline specification involves extrapolating scientific principles from model organisms, most notably the mouse. However, recent work using next generation sequencing, gene editing and differentiation of germline cells from pluripotent stem cells has revealed that the core molecular mechanisms that regulate human germline development are different from rodents. Here, we will discuss the major molecular pathways required for human germline differentiation and how pluripotent stem cells have revolutionized our ability to study the earliest steps in human embryonic lineage specification in order to understand human fertility.

1. Human reproduction

The United Nations Populations Division estimates that there are >7 billion people alive on earth today. By the middle of this century, it is estimated that the human population will reach 9 billion. At face value, these numbers suggest that the biology of human reproduction is sound. However, the United Nations Department of Economic and Social Affairs has signaled that the human population is in fertility decline, with a clear trend towards fewer children born per woman. Furthermore, the United States Centers for Disease Control and Prevention estimates that 12% of the reproductive age population (aged 15–44 years) has difficulty getting pregnant, or carrying a baby to term (CDC, 2012). Therefore, fertility decline from the point of view of population growth is most likely due to a combination of improved access to contraceptive methods, education and outreach, together with a stable but relatively high incidence of infertility. Therefore, we argue that studying the biology of human reproduction, and uncovering cell and molecular causes of human infertility is of paramount importance to human health, and the wellbeing of society.

2. Human germ cells

Infertility is caused by a range of health problems, including underlying genetic mutations, cancer, obesity, hormonal imbalance, structural malformations of the urogenital tract or injury. However, a lack of germline cells guarantees infertility because only the germline is capable of transmitting genetic and epigenetic information from parent to child. Similarly a reduction in the quality or number of germ cells produced by an individual could also have a significant impact on a person's fertility, as well as child health in the next generation.

In humans, the pioneering germ cells in the embryo are called primordial germ cells (PGCs). These primitive embryonic cells are responsible for making the entire human germline, therefore the appropriate specification and allocation of PGCs is critical to promoting human reproductive health. PGCs develop very early in embryonic life, and are first observed at around 21 days post-fertilization, with the newly specified PGCs called “early PGCs” (Fig. 1).

Once specified, early PGCs are committed and have only one fate – that is to become either oogonia that differentiate into oocytes in girls, or spermatogonia, that differentiate into mature sperm in boys. Studies of monozygotic monoamniotic twins where the incidence of discordant primary ovarian insufficiency is high, lends support to the hypothesis that the window of PGC specification in humans is very narrow (Silber et al., 2008). Monozygotic monoamniotic twins are created by embryo splitting in the peri-implantation period after the formation of the

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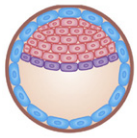
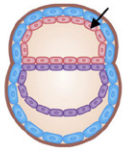
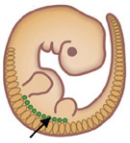
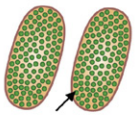
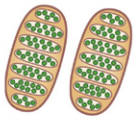
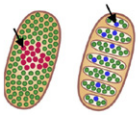
Major events in PGC development	Pre-implantation	Peri-implantation	Specified PGCs	Migrating PGCs	Colonizing PGCs	Sex Determination	PGC Differentiation
	 Blastocyst	 Amnion	 Early PGCs	 Early PGCs	 Late PGCs	 Somatic cells differentiate	 Advanced PGCs
Human (PF)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8+
Human (G)	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 10+
Mouse (E)	3.5	5.5	7.25	9.5	10.5-11.5	12.5	13.5
Macaque (PC)	Week 1	Week 2	Week 3	Week 4	Week 4	Week 5	Week 7+
Carnegie	1-4	5-6	7-9	10-13	14-15	16-17	23+

Fig. 1. Time line of PGC development in humans. Early PGCs (green) are identified in the yolk sac followed by the hindgut and then ultimately the genital ridge. Once PGCs exit the hindgut and begin expressing VASA they are called "late PGCs". Late PGCs begin to colonize the genital ridges at the start of week 5. Advanced PGCs develop at the conclusion of the Carnegie stages from 60 to 77 days with the emergence of male and female-specific transcriptional programs. In humans development is sometimes referred to as gestation (G), which refers to time since last menstrual cycle. PF = post fertilization, E = embryonic day, PC = post-coitus. The timing of mouse and macaque (rhesus) PGC development is shown for comparison.

amniotic sac. In these women, it is speculated that one twin receives the majority of PGC precursors and will have normal fertility, while the other twin will be deficient in PGC precursors, and will therefore become infertile. Put another way, once the window for germline potential has passed, the embryo cannot specify new germ cells and infertility is guaranteed.

3. Specification of the mammalian germ cell lineage is an inductive process

In model organisms such as *Drosophila, C.elegans, Xenopus* and *Danio* (zebrafish), PGCs are created each generation through a process known as pre-formation. This is a process driven by RNAs and proteins inherited from the oocyte, that selectively control translation of RNAs to endow a small number of transcriptionally quiescent cells in the embryo with PGC fate (Extavour and Akam, 2003). In contrast in mice, germ cell formation is induced by growth factor signaling from adjacent tissues leading to the expression of transcription factors that establish PGC fate.

In the mouse, elegant lineage tracing and transplantation studies have shown that mouse PGCs are specified by bone morphogenetic protein 4 (BMP4) signaling from the extra-embryonic ectoderm to a Wnt3-primed proximal posterior epiblast at around embryonic (E) day E6.25 (Fig. 2). Lineage restricted PGCs are formed from these precursors during the next 24 h, around the late primitive streak/no-bud (LS/0B) to the early bud (EB) stage of mouse embryo development (E7.25) (Kurimoto et al., 2008; Lawson et al., 1999; Lawson and Hage, 1994; Vincent et al., 2005). The transcription factor network that controls mouse PGC specification downstream of BMP4 involves three transcription factors called transcriptional repressor PR domain 1 (Prdm1), Prdm14 and Transcription Factor AP-2 gamma (Tfap2c) (Kurimoto et al., 2008; Magnusdottir et al., 2013; Vincent et al., 2005; Weber et al., 2009; Yamaji et al., 2008). Using mouse pluripotent stem cells, PRDM14 can induce PGCs directly from epiblast-like cells in the absence of BMP4 signaling (Nakaki et al., 2013). More recently, the transcription factor NANOG was also found to induce PGC formation in the absence of BMP4 (Murakami et al., 2013). The model proposed from this work follows that NANOG functions upstream of both PRDM14 and PRDM1 by binding to their enhancers in the germline-competent *in vitro* epiblast-like cells to promote PGC fate (Murakami et al., 2013).

Prior to BMP4 induction, the proximal epiblast must become responsive to PGC specification through the actions of Wnt3 (Fig. 2). However, Wnt3 acts not only to prime PGC fate, but also to regulate primitive streak formation (Liu, 1999). For example, deletion of Wnt3 or β-catenin in the mouse abolishes primitive streak formation, and also disrupts induction of *Prdm1* positive PGCs (Liu, 1999; Ohinata et al., 2009). A critical transcription factor that acts downstream of Wnt3 is Brachyury. Deleting *Brachyury* also leads to defects in primitive streak formation, and a failure to generate Prdm1 positive PGCs (Aramaki et al., 2013; Beddington et al., 1992). The relationship between Wnt3, Brachyury and BMP is complex, with the current model suggesting that Brachyury functions downstream of Wnt3 by directly inducing *Prdm1*, but only in the presence of BMP4 (Fig. 2). Without BMP4, Brachyury is still induced downstream of Wnt3 in the epiblast, however it is unable to induce expression of *Prdm1* or *Prdm14* to promote PGC fate. The one way that BMP4 can be rendered unnecessary is with the *in vitro* stem cell model using Epiblast like cells, where Brachyury can be forced into the nucleus where it induces *Prdm1* and *Prdm14* in the absence of BMP4.

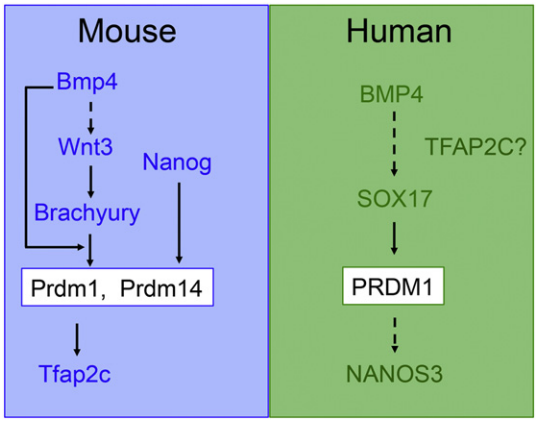


Fig. 2. Major signaling pathways and transcription factors in mouse and human PGC specification. The mouse has been invaluable for identifying the signaling pathways required for PGC specification. The finding that NANOG can induce PGCLC formation independent from BMP4 was discovered using *in vitro* differentiation into epiblast-like cells followed by induction of PGCLCs. Although the information on human PGC development is limited, initial experiments using pluripotent stem cell differentiation indicate that the mechanisms of human PGC development are different from the mouse.