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Gametogenesis: A Journey from Inception to Conception

Hailey Larose^{*,1}, Adrienne Niederriter Shami^{*,1}, Haley Abbott^{*,2}, Gabriel Manske¹, Lei Lei^{†,2,3}, Saher Sue Hammoud^{†,1,4,5}

¹Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI

²Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI

³Department of Biomedical Engineering, University of Michigan Medical School, Ann Arbor, MI

⁴Department of Obstetrics and Gynecology, University of Michigan Medical School, Ann Arbor, MI

⁵Department of Urology, University of Michigan Medical School, Ann Arbor, MI

Abstract

Gametogenesis, the process of forming mature germ cells, is an integral part of both an individual's and a species' health and well-being. This chapter focuses on critical male and female genetic and epigenetic processes underlying normal gamete formation through their differentiation to fertilization. Finally, we explore how knowledge gained from this field has contributed to progress in areas with great clinical promise, such as in vitro gametogenesis.

Keywords

gametogenesis; germ cells; oogenesis; spermatogenesis; sex determination; early embryo development; epigenetics; reprogramming

Section 1: Germ cell specification and gonadal morphogenesis

Gametes serve as a link between the past, present, and future of a species. Unlike somatic cells, the germ cells undergo a series of mitotic and meiotic divisions, followed by a differentiation program giving rise to either sperm or egg. These two mature gametes come together at the process of fertilization to produce the totipotent zygote, from which all somatic lineages/tissue and the next generation of gametes will later arise. This cycle continues indefinitely from one generation to the next, highlighting the “immortality” of the germ line. Therefore, understanding the mechanisms and pathways of cell germ cell specification, formation, and differentiation is pivotal for understanding human reproduction, infertility, and evolution.

Correspondence should be addressed to: hammou@med.umich.edu or leile@med.umich.edu.

*Denotes Equal First Authors

†Denotes co-corresponding authors

1.1 PGC specification and migration

In metazoans, primordial germ cells (PGC) are the progenitors for both male and female gametes, giving rise to spermatozoa and oocytes, respectively. Two distinct mechanisms for PGC specification have been described previously. In invertebrates (*C. elegans*, *Drosophila*) and non-mammalian vertebrates (Zebrafish and *Xenopus*), PGCs are specified by a set of maternally inherited factors, known as the germ plasm, which is comprised of RNA, proteins, and organelles that are amassed in a specific location within the oocytes, and subsequently allocated to a few cells in the embryo founding the germline (Matova & Cooley, 2001). Whereas, in mammals the precursors of PGCs arise at about embryonic day 6 (E6) from the equipotent epiblast in response to BMP signals emanating from the extraembryonic ectoderm (BMP4 and 8b) and visceral endoderm (BMP2) (Coucouvani & Martin, 1999; de Sousa Lopes et al., 2004; Lawson et al., 1999; Loebel, Watson, De Young, & Tam, 2003; McLaren, 2003; Y. Ohinata et al., 2009; Saitou, Barton, & Surani, 2002; Ying, Liu, Marble, Lawson, & Zhao, 2000). BMP signaling is indispensable for PGC specification, and targeted disruption of *Bmp2*, *Bmp4*, *Bmp8b*, or BMP signaling transducers *Smad1*, *Smad4*, *Smad5* or *Alk2*, all demonstrate loss or reduced numbers of PGCs (Lawson et al., 1999; Saitou et al., 2002; Saitou & Yamaji, 2012; Tremblay, Dunn, & Robertson, 2001; Ying et al., 2000; Ying & Zhao, 2001). The earliest known marker of nascent PGCs in mice is *Fragilis* (a member of the interferon-(IFN)-inducible transmembrane protein family) (Saitou et al., 2002). Interestingly, *Fragilis* knock-out mice do not exhibit any discernible fertility or developmental defects (Lange et al., 2008). Of the *Fragilis* positive cells, approximately only six cells will express *Blimp1* (B-lymphocyte-induced maturation protein 1, also known as *Prdm1*) and two additional key transcription factors, *Prdm14* (PR domain-containing protein 14) and *Tcfap2c* (transcription factor AP-2, gamma). *Blimp1*, *Prdm14* and *Tcfap2c* form a tripartite transcription factor network that facilitates mouse PGC specification by suppressing somatic gene expression such as *Hoxa1*, *Hoxb1*, *Lim1*, *Evx1*, *Fgf8* and *Snail* genes, while initiating the germ cell transcriptional program and triggering genome-wide epigenetic reprogramming (Figure 1) (Ancelin et al., 2006; K Hayashi, Chuva de Sousa Lopes, & Surani, 2007; Kurimoto, Yamaji, Seki, & Saitou, 2008; Magnusdottir et al., 2013; Richardson & Lehmann, 2010; Saitou & Yamaji, 2012; S. D. Vincent et al., 2005; Yamaji et al., 2008). Knockout of any of the three factors result in defects in PGC specification process. In contrast, overexpression of these three factors together in competent epiblast like cells *in vitro* is sufficient to induce mouse germ cell formation in the absence of cytokines (Magnusdottir et al., 2013), further underscoring the importance of these three transcription factors in germ cell formation and maintenance. From ~E7 onwards, the specified PGCs express the PGC-characteristic markers tissue non-specific alkaline phosphatase (TNAP), stage specific embryonic antigen 1 (SSEA1) and developmental pluripotency associated 3 (DPPA3 or STELLA) (Chiquoine, 1954; Fox, Damjanov, Martinez-Hernandez, Knowles, & Solter, 1981; Ginsburg, Snow, & McLaren, 1990; Saitou et al., 2002; Sato et al., 2002; Tam & Zhou, 1996). Interestingly, PGCs also maintain the expression of several pluripotent genes, such as SRY (sex-determining region-Y)-box2 (*Sox2*), *Nanog* and *Oct 4* (Avilion et al., 2003; Chambers et al., 2007; H. R. Schöler, Hatzopoulos, Balling, Suzuki, & Gruss, 1989; Hans R. Schöler, Ruppert, Suzuki, Chowdhury, & Gruss, 1990; Yamaguchi, Kimura, Tada, Nakatsuji, & Tada, 2005).

Although the mechanism of PGC specification in mammals is best understood in mice, additional studies in non-rodent mammals in recent years have identified similarities and differences between species. In humans, PGCs are first formed around the third week of gestation. *In vitro* models of human PGC specification from naïve pluripotent stem cells suggest that human PGCs originate from mesodermal precursor cells, and rely on BMP and WNT signaling pathways (Irie et al., 2015; Kojima et al., 2017; Tang, Kobayashi, Irie, Dietmann, & Surani, 2016). The PGC-competent human epiblast cells activate the expression of eomesodermin (*EOMES*), which subsequently induces expression of transcription factor AP2-gamma (*TFAP2C*), SRY-box 17 (*SOX17*), and *BLIMP1* concomitantly (Irie et al., 2015; Pastor et al., 2018; Tang et al., 2016) (Figure 1). Unlike mouse PGCs, hPGCs lack *Prdm14* and *SOX2* expression (Irie et al., 2015), therefore, these subtle differences in mouse and human PGC transcriptional network circuitry may be attributed to the differences in embryonic origin or in pluripotency circuitry.

Once the PGCs are specified, they proliferate while migrating through the hind gut, and then subsequently into the future gonad (also known as the genital ridge) between ~ E7.5 to E10.5 (Anderson, Copeland, Schöler, Heasman, & Wylie, 2000; Molyneaux, Stallock, Schaible, & Wylie, 2001; Richardson & Lehmann, 2010). PGC expansion and directional migration is facilitated by two germ cell – soma signaling pathways: cKIT-STEEL and SDF-CXCR4. In mice, c-KIT is expressed in germ cells, whereas, STEEL is expressed in somatic cells lining the route to the gonad. The cKIT – STEEL interaction is required for PGC proliferation, survival, and migration from the primitive streak to the future hindgut and then to the genital ridge (Ewen, Baker, Wilhelm, Aitken, & Koopman, 2009; Y. Gu, Runyan, Shoemaker, Surani, & Wylie, 2009; Ohta, Tohda, & Nishimune, 2003; Erez Raz, 2004; Runyan et al., 2006). Homozygous *cKit* or *Steel* mutant mice are sterile because they lack spermatogonial stem cells and thus differentiated germ cells (Blume-Jensen et al., 2000; Chabot, Stephenson, Chapman, Besmer, & Bernstein, 1988; Ohta et al., 2003; Reith et al., 1990). The directionality of PGC migration is also facilitated by the chemoattractant stromal cell-derived factor 1 (SDF-1) expressed at the genital ridges in the surrounding mesenchyme, which is detected by its receptor C-X-C chemokine receptor type 4 (CXCR4) expressed on the surface of PGCs (E. Raz, 2004). In support of this relationship, genetic mouse models demonstrated that removal of either SDF1 or CXCR4 resulted in very few PGCs reaching the genital ridge. Similarly, ectopic expression of SDF1 causes PGCs to migrate to new locations (Ara et al., 2003; Molyneaux et al., 2003), accounting for the development of some extra-gonadal germ cell tumors in humans (Richardson & Lehmann, 2010).

During this active migration, PGCs continue to proliferate, reaching approximately 500 PGCs in each fetal gonad at E10.5 (Pepling & Spradling, 1998). Once in the gonad, PGCs are now referred to as oogonia in females or gonocytes in males. These fetal germ cells undergo approximately five additional mitotic divisions from E10.5 to E14.5 with incomplete cytokinesis to form germline cysts (Lei & Spradling, 2013). Cysts cluster together and form germ cell nests in both female and male fetal gonads (Lei & Spradling, 2013; Mork et al., 2012; Pepling & Spradling, 1998). These nests eventually resolve and give rise to primary oocytes and gonocytes in the respective differentiated gonads.

1.2 Gonadogenesis and Sex determination

In mice, the future gonad first appears between E10 and 10.5 as regional thickenings of the epithelium overlying the ventromedial surfaces of the mesonephros. Through the use of numerous loss-of-function mouse models many genes including Lim homeobox 9 (*Lhx9*), Empty spiracles homeobox 2 (*Emx2*), Wilms tumor 1 (*Wt1*), Chromobox 2 (*Cbx2*), Nuclear receptor superfamily 5 group A (*Nr5a1*), Six homeobox (*Six1/4*), and genes encoding the insulin receptors have been implicated in establishing the bipotential genital ridges in both male and females (Birk et al., 2000; Fujimoto et al., 2013; Katoh-Fukui et al., 1998; Kreidberg et al., 1993; X. Luo, Ikeda, & Parker, 1994; Miyamoto, Yoshida, Kuratani, Matsuo, & Aizawa, 1997; Nef et al., 2003). This bipotential gonad remains morphologically and molecularly indistinguishable until sex determination, when the supporting somatic cells commit to ovarian or testis lineages (Brennan & Capel, 2004; DeFalco & Capel, 2009). Consistently, many genes involved in establishing sexual dimorphism, such as *Dax1* (dosage-sensitive sex reversal-congenital adrenal hypoplasia critical region on the X chromosome protein 1), *Sox9*, *Fgf9* and *Wnt4* are initially expressed in both XX and XY gonads, but later become developmentally restricted (Hoyle, Narvaez, Alldus, Lovell-Badge, & Swain, 2002; Y. Kim et al., 2006; Morais da Silva et al., 1996; Schmahl, Kim, Colvin, Ornitz, & Capel, 2004; Swain, Narvaez, Burgoyne, Camerino, & Lovell-Badge, 1998; Vainio, Heikkila, Kispert, Chin, & McMahon, 1999).

In males, testis morphogenesis is initiated at E11.5 when the testis determining factor SRY is first expressed in the center of the XY genital ridges (Gubbay et al., 1990; Hawkins et al., 1992; Koopman, Gubbay, Vivian, Goodfellow, & Lovell-Badge, 1991; Lovell-Badge & Robertson, 1990), and subsequently expands towards the gonadal poles (Albrecht & Eicher, 2001; Bullejos & Koopman, 2005). The expression of SRY in the supportive somatic cells specifies these cells to differentiate into SOX9⁺ Sertoli cells, which in turn proliferate in response to FGF9 (Chaboissier et al., 2004; Yuna Kim et al., 2006; Palmer & Burgoyne, 1991; Schmahl et al., 2004; Willerton, Smith, Russell, & Mackay, 2004). Interestingly, SRY and SOX9 appear to regulate a significant number of shared targets (Y. Li, Zheng, & Lau, 2014), consistent with the earlier genetic finding demonstrating that ectopic expression of SOX9 is necessary and sufficient for testis differentiation (Chaboissier et al., 2004; Vidal, Chaboissier, de Rooij, & Schedl, 2001). However, in the absence of SRY, FGF9 or SOX9, granulosa cells are specified and ovarian development is initiated in XY males (Capel, 2006; Chaboissier et al., 2004; Colvin, Green, Schmahl, Capel, & Ornitz, 2001; Jeays-Ward, Dandonneau, & Swain, 2004; Jeays-Ward et al., 2003; Kent, Wheatley, Andrews, Sinclair, & Koopman, 1996; Koopman, 1999; Koopman et al., 1991; Morais da Silva et al., 1996; Schmahl et al., 2004; Vainio et al., 1999). During normal male testis development and shortly after *Sox9* activation, the Sertoli cells begin to aggregate around clusters of germ cells and form testis cords (Combes et al., 2009). The testis cords are a hallmark structure that separates Sertoli cells and germ cells from the interstitium (Brennan & Capel, 2004). The testis embryonic interstitium arises from two cellular precursors migrating into the gonad from either the coelomic epithelium or the mesonephros in response to AMH (Karl & Capel, 1998; Martineau, Nordqvist, Tilmann, Lovell-Badge, & Capel, 1997; Ross, Tilman, Yao, MacLaughlin, & Capel, 2003). The interstitium is comprised of the steroidogenic fetal Leydig cells, peritubular myoid cells, macrophages, vasculature, and other more poorly

characterized cell types such as fibroblasts and vascular associated cells (Brennan & Capel, 2004; DeFalco, Bhattacharya, Williams, Sams, & Capel, 2014).

Female sex differentiation was originally thought to be the default pathway, during which supportive somatic cells differentiate into pre-granulosa cells. However, it is now clear that ovarian differentiation is a coordinated event driven by secreted factors including R-spondin1 (RSPO1), wntless-related MMTV integration site 4 (WNT4), and Follistatin, as well as transcriptional regulators such as β -catenin (Biaison-Laubier et al., 2007; Biaison-Laubier, Konrad, Navratil, & Schoenle, 2004; Chassot et al., 2008; Crisponi et al., 2001; Pailhoux et al., 2001; Pailhoux et al., 2002; Parma et al., 2006; Tomizuka et al., 2008; Vainio et al., 1999). In mice, RSPO and WNT4 are expressed in XX gonads from E11.5 onwards, and actively suppress testis vasculature formation, steroidogenic cell migration from the mesonephros, and germ cell loss (Jeays-Ward et al., 2003; Nicol & Yao, 2014). Global loss-of-function models of WNT4 or RSPO1 suggest both factors work through a common signaling pathway, and loss of either factor leads to testis formation in females (Chassot et al., 2008; Tomizuka et al., 2008; Vainio et al., 1999). Duplication of part of human chromosome 1p, which includes the *Wnt4* gene, was shown to lead to XY male to female sex reversal (Jordan et al., 2001). Similarly, genetic loss of the downstream targets of WNT4 and RSPO, such as β -catenin or Follistatin, results in testis development in XX gonads as well (Yao et al., 2004). However, upon normal differentiation of the fetal ovary, nested germ cells and pre-granulosa cells organize into an ovigerous cord structure – a structure more pronounced in the fetal ovary of large mammals, such as sheep and humans (Wilhelm, Palmer, & Koopman, 2007; PMID 3693081). Surrounding the ovigerous cords is an interstitium comprised of theca cells and poorly defined stromal cells that arise from either WT1+ endogenous cells of the ovary or GLI+ mesenchymal cells migrating from the mesonephros (C. Liu, Peng, Matzuk, & Yao, 2015; Martineau et al., 1997).

Although somatic cells in the fetal gonad commit to female or male lineages during sex differentiation, the somatic sex differences require active maintenance. In males, Doublesex - also known as DMRT1 - is expressed in both Sertoli cells and germ cells in adult testis. Loss of *Dmrt1* in Sertoli cells leads to the trans-differentiation of Sertoli cells to granulosa cells. Furthermore, ectopic expression of *Dmrt1* in the ovary resulted in morphological changes that are reminiscent of an ovary to testis transdifferentiation (Lindeman et al., 2015). Similarly, the FOXL2 transcription factor maintains granulosa cell fate in adult ovaries. Loss of *Foxl2* in the adult granulosa cells results in granulosa to Sertoli cell transdifferentiation (Uhlenhaut et al., 2009). Thus, it has been proposed that specification and maintenance of the male and female gonadal somatic cells is an active and constant balance between active female (*Wnt4/Rspo/B-catenin/Foxl2*) and male (*Fgf9/SRY/Sox9*) gene regulation (Chassot et al., 2008).

Section 2: Germ cell Sex determination

As somatic cells commit to testis or ovary lineages, germ cells also undergo sex differentiation in the fetal gonad and become developmentally restricted (Adams & McLaren, 2002). Meiotic entry is generally considered as a hallmark of female sex differentiation. In mice, female germ cells begin to enter meiosis at ~E13.5, and progress

through leptotene, zygotene, and pachytene stages, and become arrested at the diplotene stage between E17.5 and P5 (Borum, 1961). In the male gonad ~E14.5, gonocytes commit to male fate and exit the cell cycle, arresting at G0, and remain quiescent until after birth (Western, Miles, van den Bergen, Burton, & Sinclair, 2008).

In mammals, meiotic competency and entry appears to be regulated both intrinsically and extrinsically. Deleted in azoospermia-like (DAZL) is a key intrinsic factor enabling the PGCs to respond to cues from the somatic environment and commit to gametogenesis. DAZL is an RNA binding protein required for meiotic chromosome condensation and meiotic prophase protein expression (Y. Lin & Page, 2005). The *Dazl* mRNA is detected in both female and male germ cells between E10.5 and E11.5 (Seligman & Page, 1998). *Dazl* deficient mice are infertile due to germ cell differentiation defects (Y. Lin & Page, 2005; Ruggiu et al., 1997; Saunders et al., 2003; Schrans-Stassen, Saunders, Cooke, & de Rooij, 2001).

A well-established extrinsic regulator of meiotic entry in both male and females is retinoic acid (RA). In females, RA is produced by the fetal ovary somatic cells and the mesonephros (early nephrogenic somatic tissue attached to the fetal gonad that later develop into reproductive tracts) (Bowles et al., 2016; Koubova et al., 2006; Wilhelm et al., 2007). RA induces the expression of Stimulated by retinoic acid gene 8 (STRA8) and Meiotic recombination protein (REC8), which are required for meiotic DNA replication, and the subsequent processes of meiotic prophase, and for chromosome synapsis and segregation, respectively (Koubova et al., 2006). In the fetal ovary, *Stra8* expression occurs in an anterior-to-posterior wave. This is followed by anterior-to-posterior waves of expression of meiotic markers DMC1 (a meiotic recombinase) and synaptonemal complex protein 3 (SYCP3) (Bullejos & Koopman, 2004; Menke, Koubova, & Page, 2003; Yao, DiNapoli, & Capel, 2003). Mouse mutations disrupting the genes involved in recombination and mismatch repair, including *Spo11*, *Dmc1*, *Atm*, *Msh4*, *Mlh1*, and *Msh5*, result in both male and female infertility (Barlow et al., 1998; F. Baudat, K. Manova, J. P. Yuen, M. Jasin, & S. Keeney, 2000; Edelmann et al., 1999; Kneitz et al., 2000; Pittman et al., 1998; P. J. Romanienko & R. D. Camerini-Otero, 2000; K. Yoshida et al., 1998). Interestingly, in the *Stra8* mutant mouse ovary, fetal germ cells differentiate into primary oocytes without entering meiosis. These primary oocytes could develop in the adult ovary, but fail to mature properly due to meiotic defects (Dokshin, Baltus, Eppig, & Page, 2013). In the embryonic male gonad, RA is degraded by CYP26B1 produced by Sertoli cells, thereby preventing gonocytes from entering meiosis and inducing arrest at G0/G1 phase of mitosis around E14.5. However, in the postnatal testis, RA (initially provided by the surrounding niche) induces *Stra8* expression and is required for spermatogonial stem cell differentiation and meiotic progression (see germ cell soma section below) (Q. Zhou et al., 2008). Taken together, despite the differences in gametogenesis timing, the processes regulating meiotic licensing and onset may be conserved between the two sexes.

Section 3: Oogenesis

In mammalian females, primordial follicles that form during the fetal stage serve as the only source for egg production in adulthood. Oogenesis is comprised of two stages; oocyte

differentiation (embryonic) and oocyte development (post-puberty). During oocyte differentiation, PGCs differentiate into primary oocytes, which are further encapsulated by a single layer of pre-granulosa cells to form primordial follicles. During oocyte development, primordial follicles develop into mature follicles that ultimately produce a mature and fertilizable oocyte (Figure 2).

3.1 Oocyte differentiation and primordial follicle formation

In mice, oocyte differentiation takes place from E13.5 to P4. During this process, the germ cell nests fragment and ~ 20% of the germ cells differentiate into primary oocytes and assemble into primordial follicles (Figure 2). This process of primordial follicle formation is complex, requiring the presence of germ cells (McLaren, 1984), and is regulated by several transcription factors (*Fga* and *Foxl2*) and signaling pathways (*Notch*, *TGFβ*, and *Kit*) (Pepling, 2006, 2012, PMID 23831378). Interestingly, steroid hormones have been shown to play an inhibitory role in germ cell nest breakdown and primordial follicle formation. Specifically, neonatal mice or rats treated with estrogen (including synthetic estrogen), bisphenol-A (BPA), testosterone, or progesterone delayed primordial follicle formation or led to multi-oocyte follicles (MOFs) in adult ovaries (Iguchi, Fukazawa, Uesugi, & Takasugi, 1990; Iguchi & Takasugi, 1986; Iguchi, Takasugi, Bern, & Mills, 1986; Iguchi, Todoroki, Takasugi, & Petrow, 1988; Iguchi-Ariga & Schaffner, 1989; Jefferson, Couse, Padilla-Banks, Korach, & Newbold, 2002; A. Suzuki et al., 2002, PMID 17446182). The primordial follicles formed in the embryonic/neonatal phase remain quiescent until puberty, with only a few primordial follicles recruited immediately - giving rise to the so called the first wave of folliculogenesis (Cordeiro et al., 2015; Kerr, Myers, & Anderson, 2013).

A phenomenon unique to mammalian oocyte differentiation is the extensive germ cell loss during this process. In humans, the number of germ cells in the ovary peaks at 5 months of gestation with ~3.5 million germ cells per ovary. Germ cell number decreases to ~1 million by the time oocyte differentiation is completed in the neonatal ovary (Baker, 1963). In mice, germ cell number starts to decline from E14.5. Studies from mutant mouse models suggest that the reduction in germ cell number is may be important for clearing oocytes with chromosomal abnormalities or mitochondrial defects (Bergeron et al., 1998; Flaws, Hirshfield, Hewitt, Babus, & Furth, 2001; Gawriluk et al., 2011; Greenfeld, Pepling, Babus, Furth, & Flaws, 2007; Malki, van der Heijden, O'Donnell, Martin, & Bortvin, 2014; Perez et al., 1999; Ratts, Flaws, Kolp, Sorenson, & Tilly, 1995)). However, a study by Lei and Spradling suggests that germ cell loss is a consequence of the “nursing process” within the germline cyst during oocyte differentiation. This work showed that ~20% of the E14.5 germ cells in cysts collect cytoplasmic content and become primary oocytes, whereas the remaining 80% of the germ cells undergo apoptosis preferentially after donating their cytoplasm (L. Lei & Spradling, 2016). Which fetal germ cells become primary oocytes and what determines the number of primary oocyte during oocyte differentiation are interesting open questions.

3.2 Primordial follicle activation

To maintain normal ovarian function, quiescent primordial follicles are continually recruited to developmental phase, via primordial follicle activation. About 10–30 primordial follicles

initiate development every day in mice (Faddy & Gosden, 1995). As primordial follicles initiate development, oocytes become transcriptionally and translationally active and grow in size (Moore & Lintern-Moore, 1979). Pregranulosa cells become cuboidal and begin to proliferate. The rapid proliferation of granulosa cells facilitates the growth of the follicles (Lintern-Moore & Moore, 1979). Follicles that have just initiated follicular development are called primary follicles, which are recognized structurally by a single-layer of cuboidal granulosa cells.

The precise mechanism by which quiescent primordial follicles initiate development is not well understood. An inverse relationship between the proportion of growing follicles and the size of primordial follicle pool has been observed. This correlation led to the hypothesis that primordial follicles experience inhibitory factors from neighboring primordial follicles and growing follicles (Da Silva-Buttkus et al., 2008; Gougeon & Chainy, 1987). Two oocyte extrinsic factors implicated in primordial follicle quiescence include anti-Müllerian hormone (AMH) (Durlinger et al., 2002) and FOXL2 (Schmidt et al., 2004; Uda et al., 2004). *In vitro*, ovaries treated with AMH contained 40% fewer growing follicles compared with control ovaries. Whereas, loss of FOXL2 in granulosa cells resulted in premature follicle activation and ovarian failure (Schmidt et al., 2004; Uda et al., 2004).

Additionally, regulation of primordial follicle activation is regulated intrinsically by signaling through the PI3K (phosphoinositide 3-kinase) pathway, PTEN (phosphatase and tensin homolog deleted on chromosome 10) - a negative regulator of PI3K, and various transcription factors (i.e. *Sohlh1* (spermatogenesis and oogenesis basic helix-loop-helix transcription factor), *Sohlh2*, *Nobox* (oocyte specific homeobox gene), *Lhx8* (LIM-homeobox transcription factor 8), and *Foxo3* (Forkhead box O3)). Oocyte-specific deletion of PTEN resulted in follicular depletion, leading to premature ovarian failure in young adult mice (Reddy et al., 2008). Similar phenotypes are also observed in the null mutants all of the above four oocyte-specific transcription factors (Choi, Ballow, Xin, & Rajkovic, 2008; Choi, Yuan, & Rajkovic, 2008; Pangas et al., 2006; Rajkovic, Pangas, Ballow, Suzumori, & Matzuk, 2004). Similarly in humans, mutations in *Nobox*, *Sohlh1* and *Foxl2* are associated with human premature ovarian failure (De Baere et al., 2003; Jagarlamudi & Rajkovic, 2012; Ren et al., 2015).

3.3 Follicle development, selection and oocyte maturation

Follicle development in mammals can be divided into two phases. The first phase is termed the preantral, or gonadotropin-independent phase, which is regulated by autocrine and paracrine signaling. The second phase of follicle development, termed the antral or gonadotropin-dependent phase, is characterized by the rapid proliferation and secretive activity of granulosa cells (Figure 2). The follicular fluid secreted by granulosa cells in this stage leads to the formation of the antrum in the follicle. Surrounding each developing follicle are several layers of squamous theca cells immediately outside the basal lamina of the follicle, while, interstitial stromal cells distribute in the spaces between follicles (Zelevnik, 2004).

Follicle development is under complex regulation by cytokines and hormones. In particular, the TGF- β family plays a major role in supporting early follicle development. GDF-9 and

BMP-15 are two key oocyte-secreted members of the TGF- β superfamily. GDF-9 mRNA is synthesized only in the oocytes that have reached the primary follicle stage and beyond. In agreement with this expression pattern, *Gdf9*-null female mice are sterile due to the blockage of follicle development at the primary follicle stage (Dong et al., 1996; Günesdogan & Surani, 2016). A similar follicle development defect was observed in sheep with a mutation in *Bmp15* (Chang, Brown, & Matzuk, 2002), while *Bmp15* null mutant mice are sub-fertile with ovulation defects and decreased egg quality (Yan et al., 2001). Disruptions in these genes have also been implicated in human disease. Dysregulation of GDF-9 expression in oocytes was associated with polycystic ovarian syndrome (PCOS), and mutations in *Bmp15* were found in patients with premature ovarian failure (Di Pasquale et al., 2006; Teixeira Filho et al., 2002). Additional members of the TGF- β family with roles in follicle development include activins and inhibins (Namwanje & Brown, 2016). Conditional inactivation of *Inhba* (β A subunit gene) in developing ovarian granulosa cells in mice reduced female fertility by 38%, while complete loss of *Inhba* and *Inhbb* (activins A and B) in granulosa cells caused infertility (Pangas et al., 2007).

Follicular-stimulating hormone (FSH) and Luteinizing hormone (LH) are two major gonadotropins that promote follicle development and oocyte maturation (Williams & Erickson, 2000). Granulosa cells start to express FSH receptors at the primary follicle stage. FSH promotes granulosa cell proliferation, estrogen production, and is also required for follicle development from primary to preantral stage (Oktay, Briggs, & Gosden, 1997; Oktay, Newton, Mullan, & Gosden, 1998). LH receptors are expressed specifically in theca cells in early stage follicles and are essential for steroidogenesis (progesterone and testosterone) in theca cells. Because later stage follicle development is FSH-dependent, the fluctuation of the circulating FSH level during each menstrual cycle facilitates follicle selection. During this process, only a small fraction (one in humans) of antral follicles mature and become ovulatory follicles. According to the “FSH threshold” model, a quick elevation of FSH level due to the regression of the corpus luteum from the previous cycle allows one of the many antral follicles to quickly become the dominant follicle. The growth of the dominant follicle decreases the amount of circulating FSH due to the negative feedback. This lack of FSH causes the rest of the preantral follicles to regress (Zelevnik, 2004). Based on this mechanism of follicle selection, FSH and its’ analogs have been used to promote ovulation at IVF clinics.

During follicle development, granulosa cells and the oocyte actively communicate with each other using cellular extrusions called the trans zona pellucida. These extrusions are connected by gap junctions (connexin 43 and connexin 37) that allow small molecules to be exchanged during follicle development. In the developing follicle, the oocyte remains arrested at prophase I, which is characterized by the large nucleus, referred to as the germinal vesicle (GV). The prophase arrest is maintained via the combination of the cyclic adenosine monophosphate (cAMP) produced by the oocyte and the cyclic guanosine monophosphate (cGMP) from cumulus cells (granulosa cells that immediately surround the oocyte in the antral follicle) (PMID 16322539; PMID 27860834). The MAPK pathway, which is active in cumulus cells of the developing follicle, regulates the permeability of cyclic GMP (cGMP) from cumulus cells to the oocyte (R. Li & Albertini, 2013). In mouse preovulatory follicles, the hormone LH promotes final meiotic resumption and oocyte

maturation by stimulating a decrease in gap junction permeability and a decrease in cGMP and cAMP in the oocyte (Shuhaibar et al., 2015; PMID: 19429786). The resumption of meiosis can be recognized by the disappearance of the oocyte nucleus, namely “germinal vesicle breakdown” (GVBD) [149]. Oocytes immediately enter the second round of meiosis and arrest at metaphase by the time ovulation occurs. The second meiotic arrest is maintained by high cyclin-dependent protein kinase 1 (CDK1) activity in the oocyte and resumes only after fertilization (PMID 8015609, PMID 8015610). During meiosis, homologous chromosomes are separated during the first round of meiotic division (Meiosis I), and sister chromatids are separated during the second round of division (Meiosis II). Meiosis during oogenesis undergoes asymmetric cytokinesis. Thus, by the end of meiosis, the initial primary oocyte produces a mature oocyte and three polar bodies that will eventually degenerate. Meiosis I appears to be predisposed towards aneuploidy, especially with aging or under environmental perturbations. The surprisingly high incidence of chromosome segregation errors observed in human oocytes has been linked to defects in embryonic development and acquired birth defects such as Down syndrome (R. Li & Albertini, 2013).

Besides chromosome reduction and haploidization (so-called nuclear maturation) during oocyte maturation, the oocyte cytoplasm must also mature. Oocytes begin building their cytoplasm by collecting cytoplasmic content during oocyte differentiation at the fetal stage (nursing process). The cytoplasm expands tremendously during oocyte development through active transcription and translation (L. Lei & Spradling, 2016; Lintern-Moore & Moore, 1979). Interestingly, as oocytes resume meiosis and undergo the final stages of maturation, they become transcriptionally quiescent (Oh, Hwang, McLaughlin, Solter, & Knowles, 2000). The major translational, post-translational, and organelle modification events observed during oocyte maturation are essential for the completion of meiosis, fertilization, and early embryonic development ((Morgan et al., 2017; Oh et al., 2000; Svoboda, Franke, & Schultz, 2015)R. Li & Albertini, 2013).

Mature oocytes have a unique ability to support early embryogenesis, yet are produced in a limited number throughout a female’s reproductive life span. The drastic decline in oocyte quantity and quality in women their late 30s lead to the increased chance of infertility and birth defects. Uncovering the mechanisms underlying primordial follicle activation, as well as, the formation and maturation of oocyte cytoplasm will provide new insights in solving age-related decline in ovarian function in females.

Section 4: Spermatogenesis

The production of sperm is a continuous process throughout an individual’s lifespan, which relies on the constant supply of a rare population of cells with long-term renewal potential, called spermatogonial stem cells (SSCs), located along the basement membrane of the seminiferous tubules of the testes. In rodents, these cells are largely described by their clonal arrangement and expression of a variety of heterogeneous molecular markers. Spermatogonial cell proliferation and their ultimate differentiation into mature spermatozoa is a highly regulated developmental process, and alterations in this process result in infertility.

4.1 First wave of spermatogenesis

SSCs are derived from gonocytes, transitional cells that migrate to the basement membrane of the seminiferous tubules from the center of the testicular cords postnatally, between postnatal days 1 and 4 in mice, and 8 and 12 weeks in humans, in response to still unknown signals. This gonocyte population is heterogeneous. The portion of the gonocytes that express neurogenin 3 (*Ngn3+*) transition to form the founding SSC population in mice between postnatal days 3–6. Whereas gonocytes lacking neurogenin 3 (*Ngn-*) expression directly differentiate into A₂ spermatogonia, initiating the first wave of spermatogenesis at approximately postnatal day 3 (S. Yoshida et al., 2006; S. Yoshida et al., 2004).

The rate at which the first wave of spermatogenesis proceeds is much quicker than that of subsequent adult cycles (Ph M. Kluin, Kramer, & de Rooij, 1982). Furthermore, a significant fraction of spermatogonia and pachytene spermatocytes from the first wave undergo apoptosis (Ph M. Kluin et al., 1982; Mori et al., 1997). The apoptosis observed is mediated by caspase 3 activity and is correlated with incomplete blood testis barrier formation and not necessarily an intrinsic germ cell problem (Jahnukainen et al., 2004; Morales, Mohamed, & Cavicchia, 2007; Moreno, Lizama, Urzúa, Vergara, & Reyes, 2006). Consistent with this observation, the very few remaining first sperm produced during this pre-pubertal wave still retain the capacity to produce fertile offspring (Miki et al., 2004; S. Yoshida et al., 2006). Following this first wave of spermatogenesis, regular cycles of asynchronous sperm production begin, each lasting ~35–36 days in mouse. This highly orchestrated developmental process relies on germ cell intrinsic and extrinsic signals from somatic support cells.

4.2 Models for Spermatogenesis

The most widely accepted model for germ cell development is the “A_{single} model”. In postnatal rodent testes, the self-renewing SSCs are believed to exist in the form of a single spermatogonia (A_s). A_s SSCs divide asymmetrically to maintain the A_s pool or symmetrically to generate an A paired (A_{pr}) spermatogonia that undergo a series of synchronized division to produce A aligned (A_{al}) spermatogonia comprised of 8, 16 or 32 cells. Together, the A_s, A_{pr}, and A_{al} cells make up the total population of undifferentiated spermatogonia which is estimated to be ~ 0.3% of the total mouse testis. Only 10% of this population (or 0.03% of the testis) are A_s SSCs (Huckins, 1971; Oakberg, 1971; Phillips, Gassei, & Orwig, 2010; Valli et al., 2014).

Much effort has been invested in determining markers to specifically distinguish A_s, A_p, and A_{al} SSCs (Fayomi & Orwig, 2018). Interestingly, the majority of markers identified cover most or all undifferentiated spermatogonia (ZBTB16, SALL4, LIN28, CDH1 and FOXO1) (Buaas et al., 2004; Costoya et al., 2004; Eildermann et al., 2012; Gassei & Orwig, 2013; Goertz, Wu, Gallardo, Hamra, & Castrillon, 2011; Hobbs et al., 2012; Tokuda, Kadokawa, Kurahashi, & Marunouchi, 2007), while some were restricted to A_s spermatogonia (ID4, PAX7, BMI1) (Aloisio et al., 2014; Komai et al., 2014; M. J. Oatley, A. V. Kaucher, K. E. Racicot, & J. M. Oatley, 2011) or extend only to A_{al} (GFRA1 and NANOS2) (X. Meng et al., 2000; H. Suzuki, Sada, Yoshida, & Saga, 2009; van Bragt et al., 2008). Surprisingly, the loss of function experiments from many of the undifferentiated SSC markers, including those

most specific to A_s, show progressive infertility rather than the complete germ cell loss, suggesting there may not be a strict stem cell hierarchy (Aloisio et al., 2014; Buaas et al., 2004; Costoya et al., 2004; Eildermann et al., 2012; Fayomi & Orwig, 2018; Gassei & Orwig, 2013; Goertz et al., 2011; Hobbs et al., 2012; Komai et al., 2014; X. Meng et al., 2000; M. J. Oatley et al., 2011; H. Suzuki et al., 2009; Tokuda et al., 2007; van Bragt et al., 2008). Rather, in the absence of the ultimate SSC, other populations can compensate. From an evolutionary standpoint, a plastic stem and progenitor pool would ensure continuation of spermatogenesis, and thus fertility, even in the presence of genetic lesions.

An alternative model for germ cell development is the “fragmentation model” which relies on a series of experiments performed by the Yoshida group, who developed live-imaging to follow the behavior of spermatogonia on the basal lamina of mouse seminiferous tubules for a period of about 3 days (K. Hara et al., 2014; Kenshiro Hara et al., 2014; Nakagawa, Sharma, Nabeshima, Braun, & Yoshida, 2010). *In vivo* monitoring of *Ng3+* or *GFRα1+* single-cell dynamics and biophysical modeling of SSC kinetics during steady state or regeneration after injury suggests that the fragmented SSCs from A_{aligned} chains have the capacity to repopulate the spermatogonial niche (K. Hara et al., 2014; Toshinori Nakagawa et al., 2010). Consistent with this model, early single cell RNA sequencing studies from mice show that the undifferentiated SSC population is fairly heterogeneous and does not reveal distinct functional subtypes or hierarchy, thus, supporting a plastic stem/progenitor model rather than a hierarchal model within the undifferentiated spermatogonia (Green et al., 2018). However, it is also possible that the developmental hierarchy among the undifferentiated SPG cells may be maintained at the level of protein content or cell-cell interaction, or alternatively, is dependent on very subtle transcriptomic differences which would require much deeper sequencing to discern. Therefore, future single cell transcriptomes/proteomes on selected A_s, A_{pr}, and A_{al} cells will help reconcile the two models of SSC differentiation.

Regardless of preferred model of stem cell divisions, spermatogenesis proceeds to A_{al} (A aligned) spermatogonia which differentiate into A₁ spermatogonia upon gaining expression of the cKIT receptor, a highly conserved marker among both rodents and primates. At this stage, it is believed that the germ cells have fully committed to the complete differentiation process. Further sequential mitotic divisions produce A₂, A₃, A₄, Intermediate and B spermatogonia. The type B spermatogonia each divide to produce two primary spermatocytes and undergo meiotic reduction division to generate secondary spermatocytes which enter spermiogenesis - a process of biochemical and morphological maturation- to produce functional, flagellated spermatozoa. Consequently, due to numerous transit amplifying divisions, one mouse SSC has the potential to produce up to 4,096 sperm cells in a single spermatogenic cycle (Russell LD, 1990), although this has been shown to be a significant overestimate due to apoptosis occurring at the Type A spermatogonia stage (de Rooij, 1973; Huckins, 1978; Huckins & Oakberg, 1978; Russell, Chiarini-Garcia, Korsmeyer, & Knudson, 2002).

In humans, the SSC population does not undergo clonal expansion to the extent seen in rodents, but rather the human testis maintains a significantly larger percentage of undifferentiated spermatogonia (~22% of all cells vs. 0.3% in mice) (Paniagua, Codesal,

Nistal, Rodriguez, & Santamaria, 1987). These SSCs have been classified histologically using hematoxylin staining into A pale (A_{pale}) or A dark (A_{dark}) spermatogonia (Clermont, 1966; Yves Clermont & Michael Antar, 1973). Historically, the A_{pale} spermatogonia are thought to be the actively cycling population, while the A_{dark} acts as a reserve spermatogonia population (Clermont, 1966; Clifton & Bremner, 1983; Oakberg, 1968; van Alphen, van de Kant, & de Rooij, 1988). However, subsequent labeling studies have arrived at conflicting results (Bugeaw et al., 2005; Y. Clermont & M. Antar, 1973; de Rooij, van Alphen, & van de Kant, 1986; Ehmcke, Simorangkir, & Schlatt, 2005; Fouquet & Dadoune, 1986; P. M. Kluin, Kramer, & de Rooij, 1983; Schlatt & Weinbauer, 1994; Simorangkir, Marshall, & Plant, 2009), and whether these A_{dark} are truly quiescent or just in a different phase of the cell cycle remains to be determined. Like mice, human spermatogonia-specific markers are diverse with some overlap between species, but to date these markers do not correlate with an A dark / A pale nuclear morphology (reviewed in (Fayomi & Orwig, 2018; von Kopylow & Spiess, 2017)). Unlike rodents, the human undifferentiated spermatogonial pool undergoes only one or two mitotic divisions prior to differentiation into type B spermatogonia, entering into meiosis, and completing spermiogenesis.

4.3 Germ cell - Soma communication: genes/pathways regulating spermatogonial cell

Spermatogenesis is a continuous and asynchronous developmental process strictly controlled by the microenvironment in the seminiferous tubules. Multiple endocrine and paracrine signaling pathways have been implicated in maintaining SSC self-renewal and differentiation (Figure 3a and 3b).

GnRH-FSH-LH signaling—Gonadotropin-releasing hormone (GnRH), is a hormone produced by the hypothalamus, induce the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from gonadotrophic cells in the anterior pituitary. Both LH and FSH travel through the circulatory system to the testis where they stimulate testosterone and Gdnf production by Leydig and Sertoli cells, respectively. FSH binds to the FSH receptor in Sertoli cells, and it stimulates GDNF synthesis (see below) and stimulates Sertoli cell proliferation in the pre-pubertal testis (Dierich et al., 1998; Haywood et al., 2003; Heckert & Griswold, 2002; O'Shaughnessy, Monteiro, Verhoeven, De Gendt, & Abel, 2010). Post-puberty, FSH has been implicated in meiosis maintenance; reduction in FSH levels during meiosis resulted in increased pachytene spermatocyte apoptosis and it has been postulated that FSH may act as an apoptosis suppressor (Ruwanpura, McLachlan, Matthiesson, & Meachem, 2008). On the other hand, LHR knockout (LHRKO) males were born phenotypically normal, with testes and genital structures indistinguishable from their wild-type (WT) littermates. Postnatally, testicular growth, external genital and accessory sex organ maturation were blocked in LHRKO males, and spermatogenesis was arrested at the round spermatid stage (F. P. Zhang, Poutanen, Wilbertz, & Huhtaniemi, 2001). Furthermore, the number and size of Leydig cells were dramatically reduced. Transplanting mesenchymal stem cells into the adult testis of LHRKO mice, restored serum testosterone levels and spermatogenesis (Lo, Lei, Rao Ch, Beck, & Lamb, 2004). Despite the importance of hormonal regulation for the development of the testis and the maintenance of spermatogenesis, the molecular mechanisms through which these hormones act are still poorly understood.

GDNF-RET-GFRA1 signaling—Glial cell line–derived neurotrophic factor (GDNF) is secreted by Sertoli and peritubular myoid cells and is a well-defined paracrine factor promoting SSC renewal and maintenance. GDNF is perceived by GFRA1, a glycosylphosphatidylinositol anchored cell surface receptor for GDNF, and RET tyrosine kinase receptor within undifferentiated spermatogonia (Jing et al., 1996; C. K. Naughton, S. Jain, A. M. Strickland, A. Gupta, & J. Milbrandt, 2006). Loss of GDNF signals from Sertoli cells or peritubular myoid cells *in vivo* results in a loss of the undifferentiated germ cells, whereas overexpression leads to an expansion of the undifferentiated SSCs and the development of tumors (Chen, Willis, & Eddy, 2016; X. Meng et al., 2000). Similarly, the loss of GDNF receptors on germ cells (*RET* or *GFRA1*) phenocopies loss of GDNF ligand (Jain et al., 2004; Jijiwa et al., 2008; Cathy K. Naughton, Sanjay Jain, Amy M. Strickland, Akshay Gupta, & Jeffrey Milbrandt, 2006). Microarray analysis of cultured Thy1+ SSCs *in vitro* demonstrate that GDNF withdrawal reduces the expression of a number of transcription factors known to promote SSC self-renewal (including: *BCL6B*, *LHX1*, *ERM*, *BRACHYURY*, *ETV5*, *POU3F1* and *ID4*). Whereas, the expression of these transcription factors is restored upon addition of GDNF (Oatley, Avarbock, & Brinster, 2007; Oatley, Avarbock, Talaranta, Fearon, & Brinster, 2006; Melissa J. Oatley, Amy V. Kaucher, Karen E. Racicot, & Jon M. Oatley, 2011; S. F. Wu, Zhang, & Cairns, 2011). Through a series of molecular experiments, it has been shown that the downstream effects of GDNF signaling are mediated by PI3K/AKT-dependent pathway and the SRC family kinase (SFK) (Jijiwa et al., 2008; J. Lee et al., 2007; Oatley et al., 2007).

FGF2-MAP2K1 signaling—Fibroblast growth factor 2 (FGF2) belongs to a large family of over 15 Fgf members known to mediate epithelial-mesenchymal cell interactions (Yong Li et al., 2016). *In vitro*, FGF2 induces phosphorylation of MAPK1/3 and AKT in germ cells and stimulates the expression of *Etv5*, *Bcl6b*, and *Lhx1* genes, alongside GDNF (Ishii, Kanatsu-Shinohara, Toyokuni, & Shinohara, 2012). Mouse models have demonstrated that in FGF2-depleted testis, GDNF levels increase and SSC number increases, suggesting that a balance between FGF2 and GDNF influence SSC self-renewal and highlight the complexity in SSC regulation (Takashima et al., 2015). More research is needed to further understand how FGF2 works to regulate SSC renewal, as well as its interaction with other known SSC maintenance pathways.

Chemokine signaling—Numerous chemokines are produced by Sertoli cells, some of which have unexplored functions in spermatogenesis. One chemokine/receptor pair tested *in vitro* and *in vivo* is the C-X-C motif chemokine 12 (CXCL12) / CXCR4 receptor. CXCL12 is secreted from Sertoli cells and binds to the CXCR4 cell-surface receptor on SSCs. Studies using primary cell cultures of undifferentiated SSCs demonstrated that treatment with CXCR4-specific inhibitor results in SSC loss (Yang, Kim, Kaucher, Oatley, & Oatley, 2013). Additionally, injection of a specific CXCR4 inhibitor directly into the testis of adult mice leads to SSC loss and eventual loss of the germline (Yang et al., 2013).

Retinoic Acid (RA) signaling—Progression through spermatogenesis is regulated by intrinsic and extrinsic pulses and stage specific functions of retinoic acid (RA) signaling. Genetic and molecular studies have elegantly demonstrated that RA signaling is important

for spermatogonia differentiation, meiotic initiation, spermatid elongation, and sperm release (Van Pelt & De Rooij, 1990a, 1990b, 1991). During the first wave of spermatogenesis, RA is produced from Sertoli cells and is required spermatogonial cell differentiation (Raverdeau et al., 2012). In subsequent spermatogenic cycles, RA production becomes germ cell autonomous (Raverdeau et al., 2012), and RA signaling in different germ cell populations exerts stage-specific developmental outcomes. However, the molecular function and interactors of retinoic acid receptors (RAR) in various stages of germ cell development remains unknown.

Androgen signaling—Androgen biosynthesis occurs specifically within the Leydig cells that populate the interstitial tissue of the testis (L. Luo, Chen, Stocco, & Zirkin, 1998). Testosterone released from Leydig cells binds the *nuclear hormone androgen receptor* (AR) in Sertoli cells, peritubular myoid cells, Leydig cells, vascular smooth muscle and vascular endothelial tissue (Qing Zhou et al., 2002; X. Zhou, Kudo, Kawakami, & Hirano, 1996). Androgen signaling in Sertoli cells plays an essential role in the maintenance of the blood testes barrier (J. Meng, Holdcraft, Shima, Griswold, & Braun, 2005; Willems et al., 2010). Loss of AR signaling in Sertoli cells, as seen in the SCARKO mice, results in pachytene spermatocytes arrest (De Gendt et al., 2004; Tsai et al., 2006). Despite the importance of androgen regulation/signaling in spermatogenesis, the molecular mechanisms through which testosterone acts remains poorly understood.

In summary, germ cell development is complex process due to the reciprocal interactions between germ cells, their somatic supporting cells, and the endocrine system; all of which are required for proper sperm development and fertility. Therefore, understanding this process is not only important for potentially diagnosing and treating male infertility conditions, but also for potentially developing male contraceptive targets.

Section 5. Epigenetic Reprogramming in PGCs, gametes, and early embryos

The epigenome consists of chemical modifications on either histone proteins or DNA. The interplay between the epigenome, signal-transduction pathways, and transcription factors confers gene expression patterns in a cell, which are stably maintained in subsequent mitotic divisions. Unlike somatic cells, the sperm and egg genomes undergo two waves of epigenetic reprogramming. The first wave occurs in the embryo, but the erasure during this phase is incomplete – leaving maternally and paternally imprinted genes intact in all somatic lineages of the embryo. The second and more comprehensive wave of epigenetic reprogramming occurs upon specification of the germline to reflect the sex of the embryo (Figure 4). Despite these two global waves of epigenetic erasure, some epigenetic information can be passed from one generation to the next. However, the molecular mechanism by which epigenetic information is conveyed from one generation to the next is a long-standing biological mystery that requires elucidation.

5.1 Epigenetic reprogramming of germ cells.

PGCs arise from a small population of cells in the posterior epiblast (Magnusdottir et al., 2013; Yasuhide Ohinata et al., 2005; Weber et al., 2010; Yamaji et al., 2008). Upon specification, PGCs undergo DNA methylation erasure in two mechanistically distinct waves. The first wave is passive DNA demethylation and occurs between E6.5 and E10.5 as a result of repressing *de novo* DNA methyltransferases DNMT3a/b (Kagiwada, Kurimoto, Hirota, Yamaji, & Saitou, 2013; Kurimoto et al., 2008; Stefanie Seisenberger et al., 2012; Y. Seki et al., 2007; Shirane et al., 2016), and the rapid proliferation of PGCs. The contribution of an active DNA demethylation process during this developmental window has been ruled out since the known DNA demethylases i.e. AID, APOBEC, or TET family proteins are either not transcribed in early PGCs or their enzymatic activity is dispensable for the global decrease in DNA methylation levels noted (Kagiwada et al., 2013; Popp et al., 2010; J. J. Vincent et al., 2013). However, during this developmental time period, the maintenance DNA methyltransferase 1 (DNMT1) prevents the dilution of DNA methylation modifications on parentally imprinted regions and meiotic gene promoters (Hargan-Calvopina et al., 2016). Supporting this model, loss of *Dnmt1* in PGCs results in infertility due to premature meiotic entry in females and precocious differentiation of prospermatogonia in males (Hargan-Calvopina et al., 2016).

The second wave of demethylation takes place between E10.5 and E12.5, as PGCs migrate from the hindgut to the genital ridge and begin sex-determination. DNA methylation reaches its lowest level during this period, as the enzymes TET1 and TET2 begin to actively remove DNA methylation (Hackett et al., 2013; J. J. Vincent et al., 2013; Yamaguchi, Hong, et al., 2013). This DNA demethylation window ensures erasure of maternally/paternally imprinted loci and resistant promoter regions, leaving E13.5 germ cells with 96% of the genome hypomethylated (Kobayashi et al., 2013; Yamaguchi, Shen, Liu, Sendler, & Zhang, 2013). Surprisingly, loss of TET1 and 2 in germ cells has no effects on fertility, and only a handful of loci exhibit altered epigenetic states (Dawlaty et al., 2013; Popp et al., 2010; Yamaguchi et al., 2012; Yamaguchi, Shen, et al., 2013). These experiments indicate that the multiple families of active DNA demethylase enzymes may have redundant functions during this window.

Loss of DNA methylation in somatic lineages usually results in ectopic expression of retrotransposons, uncontrolled proliferation, and eventual cell death (Smith & Meissner, 2013). Strikingly, PGC development is not affected by the absence of DNA methylation. It has been hypothesized that the reorganization of PGC chromatin may enhance genome stability and maintain global transcriptional quiescence during the period of DNA hypomethylation. Indeed, between E8.5 and E11.5, the repressive histone marks H3 lysine 27 trimethylation (H3K27me3) and dimethylation of arginine 3 on H2A and H4 (H2A/H4R3me2) were found to increase dramatically in PGCs (Ancelin et al., 2006; Hajkova et al., 2008; Yoshiyuki Seki et al., 2005). In support of their role as modifications that prevent ectopic transcription, an early germline-specific deletion of the H3K9 methyltransferase *SETDB1* results in a loss of H3K9me3 and H3K27me3 and reactivation of the expression of retroviral elements in both sexes (S. Liu et al., 2014). Similarly, loss of *PRMT5* (the arginine methyltransferase for H2A/H4R3me2) in PGCs also causes re-expression of retroviral

elements, and results in complete sterility of both males and females (Jia Hui Ng et al., 2013).

This mechanism of germ cell reprogramming is a conserved process in multiple species. *In vivo* experiments from human PGCs have shown that the dynamics of DNA demethylation mirrors that found in rodents (Gkoutela et al., 2015; Guo et al., 2015; Tang et al., 2015). Migratory human PGCs exhibit lower levels of DNA methylation, and several weeks later, the genome of PGCs residing in the hindgut is completely hypomethylated (Guo et al., 2015; Tang et al., 2015). Imprinting regions in humans are also demethylated prior to PGCs taking up residence in the gonad, though it is not known whether this is due to lack of DNA methylation maintenance at these regions or an active DNA demethylation process seen in mice (Tang et al., 2015).

5.2 Chromatin dynamics from sex determination through gametogenesis

Most epigenetic marks are erased in PGCs by E12.5, and are reset to reflect the sex of the embryo (Figure 4). *De novo* DNA methylation in males resumes in prospermatogonia at E14.5, and is fully established at birth (Kato et al., 2007; J. Y. Li, Lees-Murdock, Xu, & Walsh, 2004). In females, DNA re-methylation does not begin until the postnatal oocyte development, where *de novo* methylation is enriched at sites of active transcription (Bourc'his, Xu, Lin, Bollman, & Bestor, 2001a; Hiura, Obata, Komiyama, Shirai, & Kono, 2006; Lucifero, Mann, Bartolomei, & Trasler, 2004; Stewart, Veselovska, & Kelsey, 2016). DNMT3A or 3B and DNMT3L have been identified as the essential factors that mediate DNA methylation at imprinted loci in both the male and female germ lines (Bestor & Bourc'his, 2004; Bourc'his & Bestor, 2004; Bourc'his, Xu, Lin, Bollman, & Bestor, 2001b; Kaneda et al., 2004). Although the sequence identity and characteristics of imprinted regions have been well characterized in gametes, the mechanism which targets the *de novo* methyltransferases to imprinting regions is still largely unexplored (Smallwood et al., 2011).

After germline specification, the next epigenetic hurdle during gametogenesis comes during meiosis. During the first meiotic division, germ cell development stalls in an extended prophase, allowing parental genomes to exchange genetic information through meiotic recombination (Marston & Amon, 2004). In this process, homologous chromosomes become paired, and large swaths of the chromosomes can be exchanged through crossover (CO) events (Kota & Feil, 2010; Marston & Amon, 2004). COs result from a complex process of DNA double-strand break (DSB) formation and repair process (Hunter, 2006). These crossover events are necessary to ensure euploidy in gametes, and its loss is implicated in infertility and aneuploidy in the offspring (Frédéric Baudat, Katia Manova, Julie Pui Yuen, Maria Jasin, & Scott Keeney, 2000; Peter J. Romanienko & R. Daniel Camerini-Otero, 2000; Zelazowski et al., 2017). The meiotic CO events occur at genomic hotspots, and are enriched at regions outside promoters that bear histone H3K4me3 peaks – established by Prdm9 (Baudat et al., 2010; Katsuhiko Hayashi, Yoshida, & Matsui, 2005; Jeffreys, Kauppi, & Neumann, 2001; Mcvean, Myers, & Hunt, 2012; S. Myers, 2005; Simon Myers, Freeman, Auton, Donnelly, & McVean, 2008; Parvanov, Petkov, & Paigen, 2010). Furthermore, histone modifications such as methylation, acetylation, ubiquitination and phosphorylation on H3, H4, H2B, and H2A.X respectively have been detected at DSB sites, respectively (Borde

et al., 2009; Buard, Barthès, Grey, & De Massy, 2009; C. S. Lee, Lee, Legube, & Haber, 2014; Yamada et al., 2004). Several mutations in enzymes involved in histone post-translational modifications (PTMs) observed in meiosis have also been shown to lead to a decrease or increase of DSB activity, suggesting a role for histone modifications in initiation/repair activity (Gong & Miller, 2013; Hirota, Mizuno, Shibata, & Ohta, 2008; Sollier et al., 2004; Yamashita, Shinohara, & Shinohara, 2004). Furthermore, the progression through meiosis is characterized by a series of transient histone variants including H1t, macroH2A, H2A.X, H2A.Z, and H3.3, all of which have been shown to have roles in the regulation of meiosis (Celeste et al., 2002; Greaves, Rangasamy, Devoy, Marshall Graves, & Tremethick, 2006; Van Der Heijden et al., 2007; B. J. Wu et al., 2014).

The chromatin reorganization that accompanies meiosis is largely transient, and the most extensive changes in chromatin state, structure or composition occurs after meiosis in both males and females (Kota & Feil, 2010). In females, histone modifications remain similar between sex determination and primordial follicle formation (between embryonic day 18.5 and postnatal day 10) (Stewart et al., 2015). Towards the later stages of oocyte development, histone 3 lysine 4 and lysine 27 methylation (H3K4me/H3K27me) marks are reorganized into broad domains (Dahl et al., 2016; X. Liu et al., 2016; B. Zhang et al., 2016; Zheng et al., 2016). The H3K4 methylation spreads out from its usual enrichment at promoters to cover more distal promoter regions and other intergenic loci (Dahl et al., 2016; X. Liu et al., 2016; B. Zhang et al., 2016). Interestingly, this non-canonical pattern of H3K4 methylation seems to function as a repressive mark in oocytes. A conditional oocyte knockout of the major H3K4 methyltransferase MLL2 resulted in defects in genome silencing, and overexpression of the H3K4me3 demethylase KDM5b re-activated transcription in quiescent, fully grown oocytes (Andreu-Vieyra et al., 2010; B. Zhang et al., 2016). In MII oocytes, H3K27me3 is present in low levels at the promoters of canonical PRC2 targets; however, the majority of H3K27me3 is instead found at intergenic regions and gene deserts with low CG density (X. Liu et al., 2016; Zheng et al., 2016). The two non-canonical chromatin modifications do not overlap, but both are found at non-transcribed regions with low levels of DNA methylation (Zheng et al., 2016). Interestingly, *de novo* DNA methylation in oocytes seems to be correlated with the patterns of chromatin modifications. Genetic manipulations which increase H3K4me3 result in impaired DNA methylation at oocyte imprinting loci (Stewart et al., 2015). The factors that control the timing and location of these chromatin marks remain unknown.

In males, post-meiotic germ cells undergo global changes in chromatin structure/packaging and the vast majority of histones in sperm are replaced with sperm-specific nuclear proteins known as protamines (Rando & Rando, 2016). The histone-to-protamine exchange is facilitated by series of intermediate steps: the hyperacetylation of histones in round spermatids, which is believed to weaken histone-DNA interactions and enabling the eviction and replacement of histones first by testes-specific histone variants, then by transition proteins, and finally by protamines (Bao & Bedford, 2016; Barral et al., 2017; Houghoughi, Barral, Vargas, Rousseaux, & Khochbin, 2018). Protamines package the sperm DNA into an endonuclease-inaccessible toroid structure that is ten-fold more compact than the heterochromatin found in somatic nuclei (Furrer, 1976; Hud, Allen, Downing, Lee, & Balhorn, 1993). The small number of histones retained in mature sperm were presumed to

be remnants of incomplete histone-to-protamine exchange; however, earlier work by us and others has demonstrated that histones are enriched at key developmental gene promoters/enhancers in mature sperm. These sperm-retained histones bear both active and repressive histone modification - a unique “poised” chromatin state termed bivalency (Brykczynska et al., 2010; Hammoud et al., 2009). Bivalency was first observed in embryonic stem cells (ESCs) (Bernstein et al., 2006), an attribute presumed to be limited to pluripotent or totipotent cells of the zygote, and important for the maintenance of the pluripotent/totipotent state. However, the presence of bivalent domains at key developmental loci suggested that competency for totipotency is already embedded in sperm chromatin (Hammoud SS 2014; Lesch, Dokshin, Young, McCarrey, & Page, 2013; J. H. Ng et al., 2013; S. Seisenberger et al., 2012). This poised chromatin state observed at developmental genes was subsequently shown to be evolutionarily conserved between the *D. rerio* and *M. musculus* male germline, spanning more than 450 million years of evolution (Arpanahi et al., 2009; Brykczynska et al., 2010; Carone et al., 2014; Erkek et al., 2013; Hammoud SS 2014; S. F. Wu et al., 2011). Therefore, the programmatic retention and evolutionary conservation of histone localization has changed our notion of the paternal contribution, and implies that epigenetic information can be passed through the paternal lineage. Furthermore, altering histone levels, or chromatin regulators during spermatogenesis leads to developmental defects that can be passed on from one generation to the next (Ihara et al., 2014; Siklenka et al., 2015). Taken together, these studies suggest that histones retained in sperm may serve as molecular carriers of epigenetic memory, but whether histones are inherited or instructive for development is not yet known.

5.3 Early embryos

Fertilization of an egg by sperm creates a totipotent zygote. During most of the first cell cycle, both the maternal and paternal haploid genomes are sequestered in separate nuclear compartments called pronuclei (Clift & Schuh, 2013). The separate genomes are replicated and epigenetically reprogrammed as the pronuclei move to meet in the middle of the single cell zygote, break down their nuclear envelopes, and unite for the embryo’s first mitosis (P G Adenot, Y Mercier, J P Renard, & E M Thompson, 1997; Clift & Schuh, 2013). Epigenetic remodeling and DNA replication occur asynchronously between the two compartments, and major epigenetic differences are observed between both pronuclei throughout the first cell cycle (Figure 4) (Puschendorf et al., 2008).

As both genomes prepare for replication, their chromatin continues to be remodeled. The maternal chromatin retains its non-canonical patterns of H3K4 and H3K27 methylation, but the H3K27me3 is specifically removed from gene promoters (B. Zhang et al., 2016; Zheng et al., 2016). Protamine proteins are immediately removed from the male pronucleus through an unknown mechanism and replaced by maternal histones. Several hours after fertilization, the paternal pronucleus is formed and enriched with acetylated maternal H3.3 histones variants and H3K27me3 at pericentromeric heterochromatin (P. G. Adenot, Y. Mercier, J. P. Renard, & E. M. Thompson, 1997; Burton & Torres-Padilla, 2014; C. J. Lin, Koh, Wong, Conti, & Ramalho-Santos, 2014; Loppin et al., 2005; Santenard et al., 2010; Tardat et al., 2015; Torres-Padilla, Bannister, Hurd, Kouzarides, & Zernicka-Goetz, 2006). The differences in chromatin landscape remain through the 8-cell-stage embryo (Ke et al., 2017).

Consistent with the differences in epigenetic landscape, allele-specific chromatin capture assays report that the maternal chromatin is highly unstructured, while the paternal chromatin displays a slightly more defined architecture (Du et al., 2017; Flyamer et al., 2017; Ke et al., 2017). It has been shown that the high-order chromatin structure in the embryo is gradually re-established during embryogenesis, and that it requires DNA replication but not zygotic genome activation (Du et al., 2017; Flyamer et al., 2017; Ke et al., 2017).

Once the male pronucleus forms, the paternal genome experiences rapid and active demethylation by the dioxygenase enzyme TET3 (T.-P. P. Gu et al., 2011; Kobayashi et al., 2012; Mayer, Niveleau, Walter, Fundele, & Haaf, 2000; Oswald et al., 2000). Active demethylation by TET3 is necessary for development, as loss-of-function mutants exhibit early embryonic lethality (T.-P. P. Gu et al., 2011). Paternally imprinted loci, and the entirety of the maternal genome, are protected from TET3 demethylation by the DNA-binding protein STELLA (Nakamura et al., 2007). This protein binds dimethylated histone H3 at lysine 9 (H3K9me2), a mark that is enriched on the maternal genome and at genomic imprinting regions (Nakamura et al., 2012). After active TET3-mediated DNA methylation, both the paternal and maternal epigenomes undergo passive demethylation throughout the remainder of the pre-implantation period (Smith et al., 2012).

During the first cell cycle, maternally-inherited factors are preparing the embryo for the transition from an egg-driven to a zygotically-controlled developmental fate (Jukam, Shariati, & Skotheim, 2017; Yartseva & Giraldez, 2015). In mice, the first major wave of transcription from the zygotic genome occurs in the late 2-cell embryo (Jukam et al., 2017). This transcription reforms canonical patterns of H3K4me3 at the promoters of actively transcribed genes in both parental alleles; however, the non-canonical patterns of H3K27me3 remain until the blastocyst stage (Dahl et al., 2016; X. Liu et al., 2016; B. Zhang et al., 2016). In general, activation of zygotic transcription begins the process of development anew. The epigenetic signatures that are acquired during the very first somatic cell fate decisions overwrite the zygote's totipotent state and restart the cycle of life.

Section 6: *In vitro* gametogenesis

Producing mature eggs or sperm using *in vitro* culture has been a research focus of reproductive biology since the early 1900s (Nagamatsu & Hayashi, 2017). Early studies of *in vitro* gametogenesis mostly focused on establishing organ culture conditions that produce mature oocytes from immature follicles or mature sperm from testicular tissue (C, 1920; Cortvrindt, Smits, & Van Steirteghem, 1996; Eppig, 1977; Eppig & O'Brien, 1996; Eppig & Schroeder, 1989; Martinovitch, 1937). By using fetal ovary organ culture, mature oocytes were acquired from cultured E12.5 mouse ovaries (Morohaku et al., 2016; Obata, Kono, & Hatada, 2002). As research techniques in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) continue to improve, recent studies have been focusing on deriving mature eggs from stem cells. The "two-step strategy" established by Hayashi et al., successfully generated mature oocytes from both ESCs and iPSCs (K. Hayashi et al., 2012; K. Hayashi, Ohta, Kurimoto, Aramaki, & Saitou, 2011; Hikabe et al., 2016). This culture strategy first induces mouse ESCs/iPSCs to epiblast-like cells by adding basic fibroblast

growth factor (bFGF) and ActivinA. Epiblast-like cells can be induced into PGC-like cells by subsequently culturing the cells with media containing BMP4, SCF, LIF and EGF (also known as 4i media) (K. Hayashi et al., 2012). PGC-like cells differentiate into oocytes by using an organ culture system, in which these cells are aggregated with somatic cells that are isolated from E12.5 fetal ovaries. ESCs/iPSC-derived MII oocytes were capable of fertilization and full-term development. However, pups were produced with 20-fold lower efficiency from ESC-derived eggs than from eggs *in vivo*, suggesting that the quality of the *in vitro*-derived oocytes is partially compromised (Hikabe et al., 2016). A similar 4i culture strategy led to the formation of human PGC-like cells (Irie et al., 2015; Sasaki et al., 2015). In addition to producing fertilizable eggs, the *in vitro* PGC differentiation system allowed scientists to identify key genes that are essential for human PGC specification, such as *Sox17*. Enforced expression of *Sox17* in human ESCs/iPSCs is sufficient for PGC-like cell specification. SOX17 acts upstream of BLIMP1 and other genes to inhibit somatic lineage and promote germline lineage differentiation (Irie et al., 2015). Recently, human oogonia was derived successfully from iPSC during a long-term (~four months) *in vitro* culture after being reconstituted with mouse fetal ovarian somatic cells. These oogonia display hallmarks of epigenetic reprogramming, such as, genome-wide DNA demethylation and imprint erasure observed during germ cell differentiation *in vivo* (Yamashiro et al., 2018).

The *in vitro* gametogenesis of male germ cells has focused on differentiating ESCs/iPSCs into spermatogonial stem cells (SSCs) and haploid cells that can fertilize eggs. The production of mature sperm from ESCs/iPSCs in culture has not been successfully achieved. Using a similar “two-step strategy”, Ishikura *et al.*, derived mouse ESCs into SSCs. In their culture system, PGC-like cells were aggregated with somatic cells dissociated from male E12.5 gonads, which reformed seminiferous tubular structures. In the reconstituted tubules, PGC-like cells differentiated into spermatogonia that express PLZF, a marker of SSCs. These spermatogonia were able to proliferate infinitely *in vitro* and produced functional sperms once transplanted into testes (Ishikura et al., 2016). Geijsen *et al.*, reported a culture system that produced haploid cells from ESCs through embryoid bodies (Geijsen et al., 2004). Later Nayernia *et al.* developed a strategy for establishing SSC lines from embryonic stem cells (Nayernia et al., 2006). These cells are able to undergo meiosis and generate haploid male gametes *in vitro*. The haploid male gametes were able to fertilize oocytes after being injected into mouse oocytes. However, the resultant offspring were not completely healthy. The offspring were either smaller or larger than controls and died between 5 days and 5 months after birth (Nayernia et al., 2006). Zhou *et al.*, reported a more robust system, in which haploid round spermatids could be derived from mouse ESCs (Q. Zhou et al., 2016). One concern has been raised is that the differentiation process was accelerated in all the culture systems. It usually takes about 6 weeks to differentiate mature sperm from PGCs via prospermatogonia *in vivo*, whereas it requires only 2–3 weeks to progress from PGC-like cells to haploid spermatid in culture (Q. Zhou et al., 2016).

Conclusion

The production of genetically (and epigenetically) competent gametes is necessary for normal fertilization and early embryonic development. Thus, understanding the underlying processes *in vivo* will allow us to better recapitulate this process *in vitro*, and potentially aid

in better defining and understanding idiopathic infertility. The research highlighted over the past few decades has not only broadened our knowledge of gamete and embryo development, and related disorders like infertility and birth defects, but also hold great promise for potentially developing novel reproductive therapies.

References

- Adams IR, & McLaren A (2002). Sexually dimorphic development of mouse primordial germ cells: switching from oogenesis to spermatogenesis. *Development*, 129(5), 1155–1164. [PubMed: 11874911]
- Adenot PG, Mercier Y, Renard JP, & Thompson EM (1997). Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. *Development*, 124(22), 4615–4625. [PubMed: 9409678]
- Adenot PG, Mercier Y, Renard JP, & Thompson EM (1997). Differential H4 acetylation of paternal and maternal chromatin precedes DNA replication and differential transcriptional activity in pronuclei of 1-cell mouse embryos. *Development (Cambridge, England)*, 124, 4615–4625.
- Albrecht KH, & Eicher EM (2001). Evidence that Sry is expressed in pre-Sertoli cells and Sertoli and granulosa cells have a common precursor. *Dev Biol*, 240(1), 92–107. doi:10.1006/dbio.2001.0438 [PubMed: 11784049]
- Aloisio GM, Nakada Y, Saatcioglu HD, Pena CG, Baker MD, Tarnawa ED, ... Castrillon DH (2014). PAX7 expression defines germline stem cells in the adult testis. *J Clin Invest*, 124(9), 3929–3944. doi:10.1172/JCI75943 [PubMed: 25133429]
- Ancelin K, Lange UC, Hajkova P, Schneider R, Bannister AJ, Kouzarides T, & Surani MA (2006). Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nature Cell Biology*, 8, 623–630. doi:10.1038/ncb1413 [PubMed: 16699504]
- Anderson R, Copeland TK, Schöler H, Heasman J, & Wylie C (2000). The onset of germ cell migration in the mouse embryo. *Mechanisms of Development*, 91(1), 61–68. doi:10.1016/S0925-4773(99)00271-3 [PubMed: 10704831]
- Andreu-Vieyra CV, Chen R, Agno JE, Glaser S, Anastassiadis K, Stewart Francis A, & Matzuk MM (2010). MLL2 is required in oocytes for bulk histone 3 lysine 4 trimethylation and transcriptional silencing. *PLoS Biology*, 8, 53–54. doi:10.1371/journal.pbio.1000453
- Ara T, Nakamura Y, Egawa T, Sugiyama T, Abe K, Kishimoto T, ... Nagasawa T (2003). Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). *Proceedings of the National Academy of Sciences*, 100(9), 5319 LP–5323.
- Arpanahi A, Brinkworth M, Iles D, Krawetz SA, Paradowska A, Platts AE, ... Miller D (2009). Endonuclease-sensitive regions of human spermatozoal chromatin are highly enriched in promoter and CTCF binding sequences. *Genome Res*, 19(8), 1338–1349. doi:gr.094953.109 [pii] 10.1101/gr.094953.109 [PubMed: 19584098]
- Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, & Lovell-Badge R (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes & Development*, 17(1), 126–140. doi:10.1101/gad.224503 [PubMed: 12514105]
- Baker TG (1963). A Quantitative and Cytological Study of Germ Cells in Human Ovaries. *Proc R Soc Lond B Biol Sci*, 158, 417–433. [PubMed: 14070052]
- Bao J, & Bedford MT (2016). Epigenetic regulation of the histone-to-protamine transition during spermiogenesis. *Reproduction*, 151, R55–R70. doi:10.1530/REP-15-0562 [PubMed: 26850883]
- Barlow C, Liyanage M, Moens PB, Tarsounas M, Nagashima K, Brown K, ... Wynshaw-Boris A (1998). Atm deficiency results in severe meiotic disruption as early as leptotema of prophase I. *Development*, 125(20), 4007–4017. [PubMed: 9735362]
- Barral S, Morozumi Y, Tanaka H, Montellier E, Govin J, de Dieuleveult M, ... Khochbin S (2017). Histone Variant H2A.L.2 Guides Transition Protein-Dependent Protamine Assembly in Male Germ Cells. *Mol Cell*, 66(1), 89–101 e108. doi:10.1016/j.molcel.2017.02.025 [PubMed: 28366643]

- Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, Przeworski M, ... de Massy B (2010). PRDM9 Is a Major Determinant of Meiotic Recombination Hotspots in Humans and Mice. *Science*, 327, 836–840. doi:10.1126/science.1183439 [PubMed: 20044539]
- Baudat F, Manova K, Yuen JP, Jasin M, & Keeney S (2000). Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Molecular Cell*, 6, 989–998. doi:10.1016/S1097-2765(00)00098-8 [PubMed: 11106739]
- Baudat F, Manova K, Yuen JP, Jasin M, & Keeney S (2000). Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol Cell*, 6(5), 989–998. [PubMed: 11106739]
- Bergeron L, Perez GI, Macdonald G, Shi L, Sun Y, Jurisicova A, ... Yuan J (1998). Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes Dev*, 12(9), 1304–1314. [PubMed: 9573047]
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, ... Lander ES (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*, 125(2), 315–326. doi:10.1016/j.cell.2006.02.041 [PubMed: 16630819]
- Bestor TH, & Bourc'h D (2004). Transposon silencing and imprint establishment in mammalian germ cells. *Cold Spring Harb Symp Quant Biol*, 69, 381–387. doi:10.1101/sqb.2004.69.381 [PubMed: 16117671]
- Biason-Laubert A, De Filippo G, Konrad D, Scarano G, Nazzaro A, & Schoenle EJ (2007). WNT4 deficiency--a clinical phenotype distinct from the classic Mayer-Rokitansky-Kuster-Hauser syndrome: a case report. *Hum Reprod*, 22(1), 224–229. doi:10.1093/humrep/del360 [PubMed: 16959810]
- Biason-Laubert A, Konrad D, Navratil F, & Schoenle EJ (2004). A WNT4 mutation associated with Mullerian-duct regression and virilization in a 46,XX woman. *N Engl J Med*, 351(8), 792–798. doi:10.1056/NEJMoa040533 [PubMed: 15317892]
- Birk OS, Casiano DE, Wassif CA, Cogliati T, Zhao L, Zhao Y, ... Westphal H (2000). The LIM homeobox gene Lhx9 is essential for mouse gonad formation. *Nature*, 403(6772), 909–913. doi:10.1038/35002622 [PubMed: 10706291]
- Blume-Jensen P, Jiang G, Hyman R, Lee KF, O'Gorman S, & Hunter T (2000). Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3'-kinase is essential for male fertility. *Nat Genet*, 24(2), 157–162. doi:10.1038/72814 [PubMed: 10655061]
- Borde V, Robine N, Lin W, Bonfils S, Géli V, & Nicolas A (2009). Histone H3 lysine 4 trimethylation marks meiotic recombination initiation sites. *EMBO Journal*, 28, 99–111. doi:10.1038/emboj.2008.257 [PubMed: 19078966]
- Borum K (1961). Oogenesis in the mouse. A study of the meiotic prophase. *Exp Cell Res*, 24, 495–507. [PubMed: 13871511]
- Bourc'h D, & Bestor TH (2004). Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature*, 431(7004), 96–99. doi:10.1038/nature02886 [PubMed: 15318244]
- Bourc'h D, Xu GL, Lin CS, Bollman B, & Bestor TH (2001a). Dnmt3L and the establishment of maternal genomic imprints. *Science*, 294(5551), 2536–2539. doi:10.1126/science.1065848 [PubMed: 11719692]
- Bourc'h D, Xu GL, Lin CS, Bollman B, & Bestor TH (2001b). Dnmt3L and the establishment of maternal genomic imprints. *Science*, 294, 2536–2539. doi:10.1126/science.1065848 [PubMed: 11719692]
- Bowles J, Feng CW, Miles K, Ineson J, Spiller C, & Koopman P (2016). ALDH1A1 provides a source of meiosis-inducing retinoic acid in mouse fetal ovaries. *Nat Commun*, 7, 10845. doi:10.1038/ncomms10845 [PubMed: 26892828]
- Brennan J, & Capel B (2004). One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet*, 5(7), 509–521. doi:10.1038/nrg1381 [PubMed: 15211353]
- Brykczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC, ... Peters AH (2010). Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nat Struct Mol Biol*, 17(6), 679–687. doi:10.1038/nsmb.1821 [PubMed: 20473313]

- Buaas FW, Kirsh AL, Sharma M, McLean DJ, Morris JL, Griswold MD, ... Braun RE (2004). Plzf is required in adult male germ cells for stem cell self-renewal. *Nat Genet*, 36(6), 647–652. doi:10.1038/ng1366 [PubMed: 15156142]
- Buageaw A, Sukhwani M, Ben-Yehudah A, Ehmcke J, Rawe VY, Pholpramool C, ... Schlatt S (2005). GDNF family receptor alpha1 phenotype of spermatogonial stem cells in immature mouse testes. *Biol Reprod*, 73(5), 1011–1016. doi:10.1095/biolreprod.105.043810 [PubMed: 16014811]
- Buard J, Barthès P, Grey C, & De Massy B (2009). Distinct histone modifications define initiation and repair of meiotic recombination in the mouse. *EMBO Journal*, 28, 2616–2624. doi:10.1038/emboj.2009.207 [PubMed: 19644444]
- Bullejos M, & Koopman P (2004). Germ cells enter meiosis in a rostro-caudal wave during development of the mouse ovary. *Mol Reprod Dev*, 68(4), 422–428. doi:10.1002/mrd.20105 [PubMed: 15236325]
- Bullejos M, & Koopman P (2005). Delayed Sry and Sox9 expression in developing mouse gonads underlies B6-Y(DOM) sex reversal. *Dev Biol*, 278(2), 473–481. doi:10.1016/j.ydbio.2004.11.030 [PubMed: 15680364]
- Burton A, & Torres-Padilla M-E (2014). Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. *Nature Reviews Molecular Cell Biology*, 15, 723–735. doi:10.1038/nrm3885 [PubMed: 25303116]
- C C (1920). Quelques résultats de la méthode de culture des tissus. *Archives de Zoologie Expérimentale et Générale*, 60, 461–500.
- Capel B (2006). R-spondin1 tips the balance in sex determination. *Nat Genet*, 38(11), 1233–1234. doi:10.1038/ng1106-1233 [PubMed: 17072299]
- Carone BR, Hung JH, Hainer SJ, Chou MT, Carone DM, Weng Z, ... Rando OJ (2014). High-resolution mapping of chromatin packaging in mouse embryonic stem cells and sperm. *Dev Cell*, 30(1), 11–22. doi:10.1016/j.devcel.2014.05.024 [PubMed: 24998598]
- Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova O. a., ... Nussenzweig A (2002). Genomic instability in mice lacking histone H2AX. *Science (New York, N.Y.)*, 296, 922–927. doi:10.1126/science.1069398
- Chaboissier MC, Kobayashi A, Vidal VI, Lutzkendorf S, van de Kant HJ, Wegner M, ... Schedl A (2004). Functional analysis of Sox8 and Sox9 during sex determination in the mouse. *Development*, 131(9), 1891–1901. doi:10.1242/dev.01087 [PubMed: 15056615]
- Chabot B, Stephenson DA, Chapman VM, Besmer P, & Bernstein A (1988). The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature*, 335(6185), 88–89. doi:10.1038/335088a0 [PubMed: 2457811]
- Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, ... Smith A (2007). Nanog safeguards pluripotency and mediates germline development. *Nature*, 450, 1230–1230. [PubMed: 18097409]
- Chang H, Brown CW, & Matzuk MM (2002). Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr Rev*, 23(6), 787–823. doi:10.1210/er.2002-0003 [PubMed: 12466190]
- Chassot AA, Ranc F, Gregoire EP, Roepers-Gajadien HL, Taketo MM, Camerino G, ... Chaboissier MC (2008). Activation of beta-catenin signaling by Rspo1 controls differentiation of the mammalian ovary. *Hum Mol Genet*, 17(9), 1264–1277. doi:10.1093/hmg/ddn016 [PubMed: 18250098]
- Chen LY, Willis WD, & Eddy EM (2016). Targeting the Gdnf Gene in peritubular myoid cells disrupts undifferentiated spermatogonial cell development. *Proc Natl Acad Sci U S A*, 113(7), 1829–1834. doi:10.1073/pnas.1517994113 [PubMed: 26831079]
- Chiquoine AD (1954). The identification, origin, and migration of the primordial germ cells in the mouse embryo. *Anat Rec*, 118(2), 135–146. [PubMed: 13138919]
- Choi Y, Ballow DJ, Xin Y, & Rajkovic A (2008). Lim homeobox gene, *lhx8*, is essential for mouse oocyte differentiation and survival. *Biol Reprod*, 79(3), 442–449. doi:10.1095/biolreprod.108.069393 [PubMed: 18509161]

- Choi Y, Yuan D, & Rajkovic A (2008). Germ cell-specific transcriptional regulator sohlh2 is essential for early mouse folliculogenesis and oocyte-specific gene expression. *Biol Reprod*, 79(6), 1176–1182. doi:10.1095/biolreprod.108.071217 [PubMed: 18753606]
- Clermont Y (1966). Renewal of spermatogonia in man. *American Journal of Anatomy*, 118, 509–524. doi:10.1002/aja.1001180211 [PubMed: 5917196]
- Clermont Y, & Antar M (1973). Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal in the monkey *Macaca arctoides*. *Am J Anat*, 136(2), 153–165. doi:10.1002/aja.1001360204 [PubMed: 4684877]
- Clermont Y, & Antar M (1973). Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal in the monkey *Macaca arctoides*. *American Journal of Anatomy*, 136, 153–165. doi:10.1002/aja.1001360204 [PubMed: 4684877]
- Clift D, & Schuh M (2013). Restarting life: Fertilization and the transition from meiosis to mitosis. *Nature Reviews Molecular Cell Biology*, 14, 549–562. doi:10.1038/nrm3643 [PubMed: 23942453]
- Clifton DK, & Bremner WJ (1983). The effect of testicular x-irradiation on spermatogenesis in man. A comparison with the mouse. *J Androl*, 4(6), 387–392. [PubMed: 6654753]
- Colvin JS, Green RP, Schmahl J, Capel B, & Ornitz DM (2001). Male-to-female sex reversal in mice lacking fibroblast growth factor 9. *Cell*, 104(6), 875–889. [PubMed: 11290325]
- Combes AN, Wilhelm D, Davidson T, Dejana E, Harley V, Sinclair A, & Koopman P (2009). Endothelial cell migration directs testis cord formation. *Dev Biol*, 326(1), 112–120. doi:10.1016/j.ydbio.2008.10.040 [PubMed: 19041858]
- Cordeiro MH, Kim SY, Ebbert K, Duncan FE, Ramalho-Santos J, & Woodruff TK (2015). Geography of follicle formation in the embryonic mouse ovary impacts activation pattern during the first wave of folliculogenesis. *Biol Reprod*, 93(4), 88. doi:10.1095/biolreprod.115.131227 [PubMed: 26246221]
- Cortvrindt R, Smitz J, & Van Steirteghem AC (1996). In-vitro maturation, fertilization and embryo development of immature oocytes from early preantral follicles from prepubertal mice in a simplified culture system. *Hum Reprod*, 11(12), 2656–2666. [PubMed: 9021369]
- Costoya JA, Hobbs RM, Barna M, Cattoretti G, Manova K, Sukhwani M, ... Pandolfi PP (2004). Essential role of Plzf in maintenance of spermatogonial stem cells. *Nat Genet*, 36(6), 653–659. doi:10.1038/ng1367 [PubMed: 15156143]
- Coucouvanis E, & Martin GR (1999). BMP signaling plays a role in visceral endoderm differentiation and cavitation in the early mouse embryo. *Development*, 126(3), 535–546. [PubMed: 9876182]
- Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, ... Pilia G (2001). The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. *Nat Genet*, 27(2), 159–166. doi:10.1038/84781 [PubMed: 11175783]
- Da Silva-Buttkus P, Jayasooriya GS, Mora JM, Mobberley M, Ryder TA, Baithun M, ... Hardy K (2008). Effect of cell shape and packing density on granulosa cell proliferation and formation of multiple layers during early follicle development in the ovary. *J Cell Sci*, 121(Pt 23), 3890–3900. doi:10.1242/jcs.036400 [PubMed: 19001500]
- Dahl JA, Jung I, Aanes H, Greggains GD, Manaf A, Lerdrup M, ... Klungland A (2016). Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature*, 537(7621), 548–552. doi:10.1038/nature19360 [PubMed: 27626377]
- Dawlaty MM, Breiling A, Le T, Raddatz G, Barrasa MI, Cheng AW, ... Jaenisch R (2013). Combined Deficiency of Tet1 and Tet2 Causes Epigenetic Abnormalities but Is Compatible with Postnatal Development. *Developmental Cell*, 24, 310–323. doi:10.1016/j.devcel.2012.12.015 [PubMed: 23352810]
- De Baere E, Beysen D, Oley C, Lorenz B, Cocquet J, De Sutter P, ... Messiaen L (2003). FOXL2 and BPES: mutational hotspots, phenotypic variability, and revision of the genotype-phenotype correlation. *Am J Hum Genet*, 72(2), 478–487. doi:10.1086/346118 [PubMed: 12529855]
- De Gendt K, Swinnen JV, Saunders PTK, Schoonjans L, Dewerchin M, Devos A, ... Verhoeven G (2004). A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. *Proceedings of the National Academy of Sciences*, 101, 1327–1332. doi:10.1073/pnas.0308114100

- de Rooij DG (1973). Spermatogonial stem cell renewal in the mouse. I. Normal situation. *Cell Tissue Kinet*, 6(3), 281–287. [PubMed: 4735543]
- de Rooij DG, van Alphen MM, & van de Kant HJ (1986). Duration of the cycle of the seminiferous epithelium and its stages in the rhesus monkey (*Macaca mulatta*). *Biol Reprod*, 35(3), 587–591. [PubMed: 3790660]
- de Sousa Lopes SM, Roelen BA, Monteiro RM, Emmens R, Lin HY, Li E, ... Mummery CL (2004). BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. *Genes Dev*, 18(15), 1838–1849. doi:10.1101/gad.294004 [PubMed: 15289457]
- DeFalco T, Bhattacharya I, Williams AV, Sams DM, & Capel B (2014). Yolk-sac-derived macrophages regulate fetal testis vascularization and morphogenesis. *Proc Natl Acad Sci U S A*, 111(23), E2384–2393. doi:10.1073/pnas.1400057111 [PubMed: 24912173]
- DeFalco T, & Capel B (2009). Gonad morphogenesis in vertebrates: divergent means to a convergent end. *Annu Rev Cell Dev Biol*, 25, 457–482. doi:10.1146/annurev.cellbio.042308.13350 [PubMed: 19807280]
- Di Pasquale E, Rossetti R, Marozzi A, Bodega B, Borgato S, Cavallo L, ... Persani L (2006). Identification of new variants of human BMP15 gene in a large cohort of women with premature ovarian failure. *J Clin Endocrinol Metab*, 91(5), 1976–1979. doi:10.1210/jc.2005-2650 [PubMed: 16464940]
- Dierich A, Sairam MR, Monaco L, Fimia GM, Gansmuller A, LeMeur M, & Sassone-Corsi P (1998). Impairing follicle-stimulating hormone (FSH) signaling in vivo: Targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *Proceedings of the National Academy of Sciences*, 95, 13612–13617. doi:10.1073/pnas.95.23.13612
- Dokshin GA, Baltus AE, Eppig JJ, & Page DC (2013). Oocyte differentiation is genetically dissociable from meiosis in mice. *Nat Genet*, 45(8), 877–883. doi:10.1038/ng.2672 [PubMed: 23770609]
- Dong et al. (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis.
- Du Z, Zheng H, Huang B, Ma R, Wu J, Zhang X, ... Xie W (2017). Allelic reprogramming of 3D chromatin architecture during early mammalian development. *Nature*, 547, 232–235. doi:10.1038/nature23263 [PubMed: 28703188]
- Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, ... Themmen AP (2002). Anti-Müllerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. *Endocrinology*, 143(3), 1076–1084. doi:10.1210/endo.143.3.8691 [PubMed: 11861535]
- Edelmann W, Cohen PE, Kneitz B, Winand N, Lia M, Heyer J, ... Kucherlapati R (1999). Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. *Nat Genet*, 21(1), 123–127. doi:10.1038/5075 [PubMed: 9916805]
- Ehmcke J, Simorangkir DR, & Schlatt S (2005). Identification of the starting point for spermatogenesis and characterization of the testicular stem cell in adult male rhesus monkeys. *Hum Reprod*, 20(5), 1185–1193. doi:10.1093/humrep/deh766 [PubMed: 15705623]
- Eildermann K, Aeckerle N, Debowski K, Godmann M, Christiansen H, Heistermann M, ... Behr R (2012). Developmental expression of the pluripotency factor sal-like protein 4 in the monkey, human and mouse testis: restriction to premeiotic germ cells. *Cells Tissues Organs*, 196(3), 206–220. doi:10.1159/000335031 [PubMed: 22572102]
- Eppig JJ (1977). Mouse oocyte development in vitro with various culture systems. *Dev Biol*, 60(2), 371–388. [PubMed: 562802]
- Eppig JJ, & O'Brien MJ (1996). Development in vitro of mouse oocytes from primordial follicles. *Biol Reprod*, 54(1), 197–207. [PubMed: 8838017]
- Eppig JJ, & Schroeder AC (1989). Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization in vitro. *Biol Reprod*, 41(2), 268–276. [PubMed: 2508774]
- Erkek S, Hisano M, Liang CY, Gill M, Murr R, Dieker J, ... Peters AH (2013). Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nat Struct Mol Biol*, 20(10), 1236. doi:10.1038/nsmb1013-1236b

- Ewen K, Baker M, Wilhelm D, Aitken RJ, & Koopman P (2009). Global Survey of Protein Expression during Gonadal Sex Determination in Mice. *Molecular & Cellular Proteomics*, 8(12), 2624–2641. doi:10.1074/mcp.M900108-MCP200 [PubMed: 19617587]
- Faddy MJ, & Gosden RG (1995). A mathematical model of follicle dynamics in the human ovary. *Hum Reprod*, 10(4), 770–775. [PubMed: 7650119]
- Fayomi AP, & Orwig KE (2018). Spermatogonial stem cells and spermatogenesis in mice, monkeys and men. *Stem Cell Res*, 29, 207–214. doi:10.1016/j.scr.2018.04.009 [PubMed: 29730571]
- Flaws JA, Hirshfield AN, Hewitt JA, Babus JK, & Furth PA (2001). Effect of bcl-2 on the primordial follicle endowment in the mouse ovary. *Biol Reprod*, 64(4), 1153–1159. [PubMed: 11259262]
- Flyamer IM, Gassler J, Imakaev M, Brandão HB, Ulianov SV, Abdennur N, ... Tachibana-Konwalski K (2017). Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. *Nature*, 544, 110–114. doi:10.1038/nature21711 [PubMed: 28355183]
- Fouquet JP, & Dadoune JP (1986). Renewal of spermatogonia in the monkey (*Macaca fascicularis*). *Biol Reprod*, 35(1), 199–207. [PubMed: 3741951]
- Fox N, Damjanov I, Martinez-Hernandez A, Knowles BB, & Solter D (1981). Immunohistochemical localization of the early embryonic antigen (SSEA-1) in postimplantation mouse embryos and fetal and adult tissues. *Developmental Biology*, 83(2), 391–398. doi:10.1016/0012-1606(81)90487-5 [PubMed: 6113181]
- Fujimoto Y, Tanaka SS, Yamaguchi YL, Kobayashi H, Kuroki S, Tachibana M, ... Nishinakamura R (2013). Homeoproteins Six1 and Six4 regulate male sex determination and mouse gonadal development. *Dev Cell*, 26(4), 416–430. doi:10.1016/j.devcel.2013.06.018 [PubMed: 23987514]
- Furrer R (1976). Physical Characteristics of. 16, 811–825. doi:10.1007/978-3-662-47337-5
- Gasse K, & Orwig KE (2013). SALL4 expression in gonocytes and spermatogonial clones of postnatal mouse testes. *PLoS One*, 8(1), e53976. doi:10.1371/journal.pone.0053976 [PubMed: 23326552]
- Gawriluk TR, Hale AN, Flaws JA, Dillon CP, Green DR, & Rucker EB 3rd. (2011). Autophagy is a cell survival program for female germ cells in the murine ovary. *Reproduction*, 141(6), 759–765. doi:10.1530/REP-10-0489 [PubMed: 21464117]
- Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, & Daley GQ (2004). Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature*, 427(6970), 148–154. doi:10.1038/nature02247 [PubMed: 14668819]
- Ginsburg M, Snow M, & McLaren A (1990). Primordial germ cells in the mouse embryo during gastrulation. *Development*, 528(2), 521–528.
- Gkoutela S, Zhang KX, Shafiq TA, Liao WW, Hargan-Calvopiña J, Chen PY, & Clark AT (2015). DNA demethylation dynamics in the human prenatal germline. *Cell*, 161, 1425–1436. doi:10.1016/j.cell.2015.05.012 [PubMed: 26004067]
- Goertz MJ, Wu Z, Gallardo TD, Hamra FK, & Castrillon DH (2011). Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis. *J Clin Invest*, 121(9), 3456–3466. doi:10.1172/JCI57984 [PubMed: 21865646]
- Gong F, & Miller KM (2013). Mammalian DNA repair: HATs and HDACs make their mark through histone acetylation. *Mutat Res*, 750(1–2), 23–30. doi:10.1016/j.mrfmmm.2013.07.002 [PubMed: 23927873]
- Gougeon A, & Chainy GB (1987). Morphometric studies of small follicles in ovaries of women at different ages. *J Reprod Fertil*, 81(2), 433–442. [PubMed: 3430463]
- Greaves IK, Rangasamy D, Devoy M, Marshall Graves JA, & Tremethick DJ (2006). The X and Y Chromosomes Assemble into H2A.Z, Containing Facultative Heterochromatin, following Meiosis. *Molecular and Cellular Biology*, 26, 5394–5405. doi:10.1128/MCB.00519-06 [PubMed: 16809775]
- Green CD, Ma Q, Manske GL, Shami AN, Zheng X, Marini S, ... Hammoud SS (2018). A Comprehensive Roadmap of Murine Spermatogenesis Defined by Single-Cell RNA-Seq. *Dev Cell*, 46(5), 651–667 e610. doi:10.1016/j.devcel.2018.07.025 [PubMed: 30146481]
- Greenfield CR, Pepling ME, Babus JK, Furth PA, & Flaws JA (2007). BAX regulates follicular endowment in mice. *Reproduction*, 133(5), 865–876. doi:10.1530/REP-06-0270 [PubMed: 17616717]

- Gu T-PP, Guo F, Yang H, Wu H-PP, Xu GLG-FFG-L, Liu W, ... Xu GLG-FFG-L (2011). The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. *Nature*, 477, 606–612. doi:10.1038/nature10443 [PubMed: 21892189]
- Gu Y, Runyan C, Shoemaker A, Surani A, & Wylie C (2009). Steel factor controls primordial germ cell survival and motility from the time of their specification in the allantois, and provides a continuous niche throughout their migration. *Development*, 136(8), 1295–1303. doi:10.1242/dev.030619 [PubMed: 19279135]
- Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, ... Lovell-Badge R (1990). A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature*, 346(6281), 245–250. doi:10.1038/346245a0 [PubMed: 2374589]
- Günesdogan U, & Surani MA (2016). Developmental Competence for Primordial Germ Cell Fate. *Current Topics in Developmental Biology*, 117, 471–496. doi:10.1016/BS.CTDB.2015.11.007 [PubMed: 26969996]
- Guo F, Yan L, Guo H, Li L, Hu B, Zhao Y, ... Qiao J (2015). The transcriptome and DNA methylome landscapes of human primordial germ cells. *Cell*, 161, 1437–1452. doi:10.1016/j.cell.2015.05.015 [PubMed: 26046443]
- Hackett JA, Sengupta R, Zyliz JJ, Murakami K, Lee C, Down TA, & Surani MA (2013). Germline DNA Demethylation Dynamics and Imprint Erasure Through 5-Hydroxymethylcytosine. *Science*, 339, 448–452. doi:10.1126/science.1229277 [PubMed: 23223451]
- Hajkova P, Ancelin K, Waldmann T, Lacoste N, Lange UC, Cesari F, ... Surani MA (2008). Chromatin dynamics during epigenetic reprogramming in the mouse germ line. *Nature*, 452, 877–881. doi:10.1038/nature06714 [PubMed: 18354397]
- Hammoud SS, L. D, Yi C, Carrell DT, Guccione E, Cairns BR. (2014). Chromatin and Transcriptional Logic in Adult Germline Stem Cells and Mammalian Spermatogenesis. *Cell Stem Cell*, In press.
- Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, & Cairns BR (2009). Distinctive chromatin in human sperm packages genes for embryo development. *Nature*, 460(7254), 473–478. doi:10.1038/nature08162 [PubMed: 19525931]
- Hara K, Nakagawa T, Enomoto H, Suzuki M, Yamamoto M, Simons BD, & Yoshida S (2014). Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states. *Cell Stem Cell*, 14(5), 658–672. doi:10.1016/j.stem.2014.01.019 [PubMed: 24792118]
- Hara K, Nakagawa T, Enomoto H, Suzuki M, Yamamoto M, Simons BD, & Yoshida S (2014). Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states. *Cell Stem Cell*, 14, 658–672. doi:10.1016/j.stem.2014.01.019 [PubMed: 24792118]
- Hargan-Calvopina J, Taylor S, Cook H, Hu Z, Lee SA, Yen M. r., ... Clark AT (2016). Stage-Specific Demethylation in Primordial Germ Cells Safeguards against Precocious Differentiation. *Developmental Cell*, 39, 75–86. doi:10.1016/j.devcel.2016.07.019 [PubMed: 27618282]
- Hawkins JR, Taylor A, Berta P, Levilliers J, Van der Auwera B, & Goodfellow PN (1992). Mutational analysis of SRY: nonsense and missense mutations in XY sex reversal. *Hum Genet*, 88(4), 471–474. [PubMed: 1339396]
- Hayashi K, Chuva de Sousa Lopes, S. M, & Surani MA (2007). Germ Cell Specification in Mice. *Science*, 316, 394–396. [PubMed: 17446386]
- Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, & Saitou M (2012). Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science*, 338(6109), 971–975. doi:10.1126/science.1226889 [PubMed: 23042295]
- Hayashi K, Ohta H, Kurimoto K, Aramaki S, & Saitou M (2011). Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell*, 146(4), 519–532. doi:10.1016/j.cell.2011.06.052 [PubMed: 21820164]
- Hayashi K, Yoshida K, & Matsui Y (2005). A histone H3 methyltransferase controls epigenetic events required for meiotic prophase. *Nature*, 438, 374–378. doi:10.1038/nature04112 [PubMed: 16292313]

- Haywood M, Spaliviero J, Jimenez M, King NJ, Handelsman DJ, & Allan CM (2003). Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone. *Endocrinology*, 144(2), 509–517. doi:10.1210/en.2002-220710 [PubMed: 12538611]
- Heckert LL, & Griswold MD (2002). The Expression of the Follicle-stimulating Hormone Receptor in Spermatogenesis. *Recent progress in hormone research*, 57, 129–148. [PubMed: 12017540]
- Hikabe O, Hamazaki N, Nagamatsu G, Obata Y, Hirao Y, Hamada N, ... Hayashi K (2016). Reconstitution in vitro of the entire cycle of the mouse female germ line. *Nature*, 539(7628), 299–303. doi:10.1038/nature20104 [PubMed: 27750280]
- Hirota K, Mizuno K, Shibata T, & Ohta K (2008). Distinct chromatin modulators regulate the formation of accessible and repressive chromatin at the fission yeast recombination hotspot ade6-M26. *Mol Biol Cell*, 19(3), 1162–1173. doi:10.1091/mbc.E07-04-0377 [PubMed: 18199689]
- Hiura H, Obata Y, Komiyama J, Shirai M, & Kono T (2006). Oocyte growth-dependent progression of maternal imprinting in mice. *Genes to Cells*, 11, 353–361. doi:10.1111/j.1365-2443.2006.00943.x [PubMed: 16611239]
- Hobbs RM, Fagoonee S, Papa A, Webster K, Altruda F, Nishinakamura R, ... Pandolfi PP (2012). Functional antagonism between Sall4 and Plzf defines germline progenitors. *Cell Stem Cell*, 10(3), 284–298. doi:10.1016/j.stem.2012.02.004 [PubMed: 22385656]
- Hoghoughi N, Barral S, Vargas A, Rousseaux S, & Khochbin S (2018). Histone variants: essential actors in male genome programming. *J Biochem*, 163(2), 97–103. doi:10.1093/jb/mvx079 [PubMed: 29165574]
- Hoyle C, Narvaez V, Alldus G, Lovell-Badge R, & Swain A (2002). Dax1 expression is dependent on steroidogenic factor 1 in the developing gonad. *Mol Endocrinol*, 16(4), 747–756. doi:10.1210/mend.16.4.0802 [PubMed: 11923472]
- Huckins C (1971). The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. *Anat Rec*, 169(3), 533–557. doi:10.1002/ar.1091690306 [PubMed: 5550532]
- Huckins C (1978). The morphology and kinetics of spermatogonial degeneration in normal adult rats: an analysis using a simplified classification of the germinal epithelium. *Anat Rec*, 190(4), 905–926. doi:10.1002/ar.1091900410 [PubMed: 637327]
- Huckins C, & Oakberg EF (1978). Morphological and quantitative analysis of spermatogonia in mouse testes using whole mounted seminiferous tubules. I. The normal testes. *Anat Rec*, 192(4), 519–528. doi:10.1002/ar.1091920406 [PubMed: 736272]
- Identification of the Elemental Packing Unit of DNA in Mammalian Sperm Cells by Atomic Force Microscopy, 193 1347–1354 (1993).
- Hunter N (2006). Meiotic recombination In Aguilera ARR (Ed.), *Molecular Genetics of Recombination* (Vol. 17). Berlin, Heidelberg: Springer.
- Iguchi T, Fukazawa Y, Uesugi Y, & Takasugi N (1990). Polyovular follicles in mouse ovaries exposed neonatally to diethylstilbestrol in vivo and in vitro. *Biol Reprod*, 43(3), 478–484. [PubMed: 2271729]
- Iguchi T, & Takasugi N (1986). Polyovular follicles in the ovary of immature mice exposed prenatally to diethylstilbestrol. *Anat Embryol (Berl)*, 175(1), 53–55. [PubMed: 3799991]
- Iguchi T, Takasugi N, Bern HA, & Mills KT (1986). Frequent occurrence of polyovular follicles in ovaries of mice exposed neonatally to diethylstilbestrol. *Teratology*, 34(1), 29–35. doi:10.1002/tera.1420340105 [PubMed: 3764775]
- Iguchi T, Todoroki R, Takasugi N, & Petrow V (1988). The effects of an aromatase inhibitor and a 5 alpha-reductase inhibitor upon the occurrence of polyovular follicles, persistent anovulation, and permanent vaginal stratification in mice treated neonatally with testosterone. *Biol Reprod*, 39(3), 689–697. [PubMed: 3196799]
- Iguchi-Arigo SM, & Schaffner W (1989). CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. *Genes Dev*, 3(5), 612–619. [PubMed: 2545524]

- Ihara M, Meyer-Ficca ML, Leu NA, Rao S, Li F, Gregory BD, ... Meyer RG (2014). Paternal Poly (ADP-ribose) Metabolism Modulates Retention of Inheritable Sperm Histones and Early Embryonic Gene Expression. *PLoS Genetics*, 10. doi:10.1371/journal.pgen.1004317
- Irie N, Weinberger L, Tang WW, Kobayashi T, Viukov S, Manor YS, ... Surani MA (2015). SOX17 is a critical specifier of human primordial germ cell fate. *Cell*, 160(1–2), 253–268. doi:10.1016/j.cell.2014.12.013 [PubMed: 25543152]
- Ishii K, Kanatsu-Shinohara M, Toyokuni S, & Shinohara T (2012). FGF2 mediates mouse spermatogonial stem cell self-renewal via upregulation of *Etv5* and *Bcl6b* through MAP2K1 activation. *Development*, 139(10), 1734 LP–1743. [PubMed: 22491947]
- Ishikura Y, Yabuta Y, Ohta H, Hayashi K, Nakamura T, Okamoto I, ... Saitou M (2016). In Vitro Derivation and Propagation of Spermatogonial Stem Cell Activity from Mouse Pluripotent Stem Cells. *Cell Rep*, 17(10), 2789–2804. doi:10.1016/j.celrep.2016.11.026 [PubMed: 27926879]
- Jagarlamudi K, & Rajkovic A (2012). Oogenesis: transcriptional regulators and mouse models. *Mol Cell Endocrinol*, 356(1–2), 31–39. doi:10.1016/j.mce.2011.07.049 [PubMed: 21856374]
- Jahnukainen K, Chrysis D, Hou M, Parvinen M, Eksborg S, & Söder O (2004). Increased Apoptosis Occurring During the First Wave of Spermatogenesis Is Stage-Specific and Primarily Affects Midpachytene Spermatocytes in the Rat Testis. *Biology of Reproduction*, 70, 290–296. doi:10.1095/biolreprod.103.018390 [PubMed: 14522836]
- Jain S, Naughton CK, Yang M, Strickland A, Vij K, Encinas M, ... Milbrandt J (2004). Mice expressing a dominant-negative Ret mutation phenocopy human Hirschsprung disease and delineate a direct role of Ret in spermatogenesis. *Development*, 131(21), 5503 LP–5513. [PubMed: 15469971]
- Jeays-Ward K, Dandonneau M, & Swain A (2004). Wnt4 is required for proper male as well as female sexual development. *Dev Biol*, 276(2), 431–440. doi:10.1016/j.ydbio.2004.08.049 [PubMed: 15581876]
- Jeays-Ward K, Hoyle C, Brennan J, Dandonneau M, Alldus G, Capel B, & Swain A (2003). Endothelial and steroidogenic cell migration are regulated by WNT4 in the developing mammalian gonad. *Development*, 130(16), 3663–3670. [PubMed: 12835383]
- Jefferson WN, Couse JF, Padilla-Banks E, Korach KS, & Newbold RR (2002). Neonatal exposure to genistein induces estrogen receptor (ER)α expression and multiocyte follicles in the maturing mouse ovary: evidence for ERβ-mediated and nonestrogenic actions. *Biol Reprod*, 67(4), 1285–1296. [PubMed: 12297547]
- Jeffreys A, Kauppi L, & Neumann R (2001). Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. *Nature genetics*, 29, 217–222. doi:10.1038/ng1001-217 [PubMed: 11586303]
- Jijiwa M, Kawai K, Fukihara J, Nakamura A, Hasegawa M, Suzuki C, ... Takahashi M (2008). GDNF-mediated signaling via RET tyrosine 1062 is essential for maintenance of spermatogonial stem cells. *Genes to Cells*, 13(4), 365–374. doi:10.1111/j.1365-2443.2008.01171.x [PubMed: 18363967]
- Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, ... Fox GM (1996). GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-α, a novel receptor for GDNF. *Cell*, 85(7), 1113–1124. [PubMed: 8674117]
- Jordan BK, Mohammed M, Ching ST, Delot E, Chen XN, Dewing P, ... Vilain E (2001). Up-regulation of WNT-4 signaling and dosage-sensitive sex reversal in humans. *Am J Hum Genet*, 68(5), 1102–1109. doi:10.1086/320125 [PubMed: 11283799]
- Jukam D, Shariati SAM, & Skotheim JM (2017). Zygotic Genome Activation in Vertebrates. *Developmental Cell*, 42, 316–332. doi:10.1016/j.devcel.2017.07.026 [PubMed: 28829942]
- Kagiwada S, Kurimoto K, Hirota T, Yamaji M, & Saitou M (2013). Replication-coupled passive DNA demethylation for the erasure of genome imprints in mice. *EMBO Journal*, 32, 340–353. doi:10.1038/emboj.2012.331 [PubMed: 23241950]
- Kaneda M, Okano M, Hata K, Sado T, Tsujimoto H, Li E, & Sasaki H (2004). Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature*, 429, 900–903. doi:10.1038/nature02633 [PubMed: 15215868]

- Karl J, & Capel B (1998). Sertoli cells of the mouse testis originate from the coelomic epithelium. *Dev Biol*, 203(2), 323–333. doi:10.1006/dbio.1998.9068 [PubMed: 9808783]
- Kato Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, ... Sasaki H (2007). Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. *Human Molecular Genetics*, 16, 2272–2280. doi:10.1093/hmg/ddm179 [PubMed: 17616512]
- Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Noguchi K, & Higashinakagawa T (1998). Male-to-female sex reversal in M33 mutant mice. *Nature*, 393(6686), 688–692. doi:10.1038/31482 [PubMed: 9641679]
- Ke Y, Xu Y, Chen X, Feng S, Liu Z, Sun Y, ... Liu J (2017). 3D Chromatin Structures of Mature Gametes and Structural Reprogramming during Mammalian Embryogenesis. *Cell*, 170, 367–381.e320. doi:10.1016/j.cell.2017.06.029 [PubMed: 28709003]
- Kent J, Wheatley SC, Andrews JE, Sinclair AH, & Koopman P (1996). A male-specific role for SOX9 in vertebrate sex determination. *Development*, 122(9), 2813–2822. [PubMed: 8787755]
- Kerr JB, Myers M, & Anderson RA (2013). The dynamics of the primordial follicle reserve. *Reproduction*, 146(6), R205–215. doi:10.1530/REP-13-0181 [PubMed: 23929903]
- Kim Y, Kobayashi A, Sekido R, DiNapoli L, Brennan J, Chaboissier MC, ... Capel B (2006). Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biology*, 4(6), 1000–1009. doi:10.1007/11558958_121
- Kim Y, Kobayashi A, Sekido R, DiNapoli L, Brennan J, Chaboissier MC, ... Capel B (2006). Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biol*, 4(6), e187. doi:10.1371/journal.pbio.0040187 [PubMed: 16700629]
- Kluin PM, Kramer MF, & de Rooij DG (1982). Spermatogenesis in the immature mouse proceeds faster than in the adult. *International Journal of Andrology*, 5, 282–294. doi:10.1111/j.1365-2605.1982.tb00257.x [PubMed: 7118267]
- Kluin PM, Kramer MF, & de Rooij DG (1983). Testicular development in *Macaca irus* after birth. *Int J Androl*, 6(1), 25–43. [PubMed: 6840878]
- Kneitz B, Cohen PE, Avdievich E, Zhu L, Kane MF, Hou H Jr., ... Edelman W (2000). MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. *Genes Dev*, 14(9), 1085–1097. [PubMed: 10809667]
- Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, Yayoi O, ... Kono T (2012). Contribution of Intragenic DNA Methylation in Mouse Gametic DNA Methylomes to Establish Oocyte-Specific Heritable Marks. *PLoS Genetics*, 8, e1002440. doi:10.1371/journal.pgen.1002440 [PubMed: 22242016]
- Kobayashi H, Sakurai T, Miura F, Imai M, Mochiduki K, Yanagisawa E, ... Kono T (2013). High-resolution DNA methylome analysis of primordial germ cells identifies gender-specific reprogramming in mice. *Genome Research*, 23, 616–627. doi:10.1101/gr.148023.112 [PubMed: 23410886]
- Kojima Y, Sasaki K, Yokobayashi S, Sakai Y, Nakamura T, Yabuta Y, ... Saitou M (2017). Evolutionarily Distinctive Transcriptional and Signaling Programs Drive Human Germ Cell Lineage Specification from Pluripotent Stem Cells. *Cell Stem Cell*, 21(4), 517–532 e515. doi:10.1016/j.stem.2017.09.005 [PubMed: 28985527]
- Komai Y, Tanaka T, Tokuyama Y, Yanai H, Ohe S, Omachi T, ... Ueno H (2014). Bmi1 expression in long-term germ stem cells. *Sci Rep*, 4, 6175. doi:10.1038/srep06175 [PubMed: 25146451]
- Koopman P (1999). Sry and Sox9: mammalian testis-determining genes. *Cell Mol Life Sci*, 55(6–7), 839–856. [PubMed: 10412367]
- Koopman P, Gubbay J, Vivian N, Goodfellow P, & Lovell-Badge R (1991). Male development of chromosomally female mice transgenic for Sry. *Nature*, 351(6322), 117–121. doi:10.1038/351117a0 [PubMed: 2030730]
- Kota SK, & Feil R (2010). Epigenetic Transitions in Germ Cell Development and Meiosis. *Developmental Cell*, 19, 675–686. doi:10.1016/j.devcel.2010.10.009 [PubMed: 21074718]
- Koubova J, Menke DB, Zhou Q, Capel B, Griswold MD, & Page DC (2006). Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc Natl Acad Sci U S A*, 103(8), 2474–2479. doi:10.1073/pnas.0510813103 [PubMed: 16461896]

- Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, & Jaenisch R (1993). WT-1 is required for early kidney development. *Cell*, 74(4), 679–691. doi:10.1016/0092-8674(93)90515-R [PubMed: 8395349]
- Kurimoto K, Yamaji M, Seki Y, & Saitou M (2008). Specification of the germ cell lineage in mice: A process orchestrated by the PR-domain proteins, Blimp1 and Prdm14. *Cell Cycle*, 7, 3514–3518. doi:10.4161/cc.7.22.6979 [PubMed: 19001867]
- Lange UC, Adams DJ, Lee C, Barton S, Schneider R, Bradley A, & Surani MA (2008). Normal germ line establishment in mice carrying a deletion of the Ifitm/Fragilis gene family cluster. *Mol Cell Biol*, 28(15), 4688–4696. doi:10.1128/MCB.00272-08 [PubMed: 18505827]
- Lawson KA, Dunn NR, Roelen BA, Zeinstra LM, Davis AM, Wright CV, ... Hogan BL (1999). Bmp4 is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev*, 13(4), 424–436. [PubMed: 10049358]
- Lee CS, Lee K, Legube G, & Haber JE (2014). Dynamics of yeast histone H2A and H2B phosphorylation in response to a double-strand break. *Nature Structural and Molecular Biology*, 21, 103–109. doi:10.1038/nsmb.2737
- Lee J, Kanatsu-Shinohara M, Inoue K, Ogonuki N, Miki H, Toyokuni S, ... Shinohara T (2007). Akt mediates self-renewal division of mouse spermatogonial stem cells. *Development*, 134(10), 1853–1859. doi:10.1242/dev.003004 [PubMed: 17428826]
- Lei L, & Spradling AC (2013). Mouse primordial germ cells produce cysts that partially fragment prior to meiosis. *Development*, 140(10), 2075 LP–2081. [PubMed: 23578925]
- Lei L, & Spradling AC (2016). Mouse oocytes differentiate through organelle enrichment from sister cyst germ cells. *Science*, 352(6281), 95–99. doi:10.1126/science.aad2156 [PubMed: 26917595]
- Lesch BJ, Dokshin GA, Young RA, McCarrey JR, & Page DC (2013). A set of genes critical to development is epigenetically poised in mouse germ cells from fetal stages through completion of meiosis. *Proc Natl Acad Sci U S A*, 110(40), 16061–16066. doi:10.1073/pnas.1315204110 [PubMed: 24043772]
- Li JY, Lees-Murdock DJ, Xu GL, & Walsh CP (2004). Timing of establishment of paternal methylation imprints in the mouse. *Genomics*, 84, 952–960. doi:10.1016/j.ygeno.2004.08.012 [PubMed: 15533712]
- Li R, & Albertini DF (2013). The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. *Nat Rev Mol Cell Biol*, 14(3), 141–152. doi:10.1038/nrm3531 [PubMed: 23429793]
- Li Y, Sun C, Yates EA, Jiang C, Wilkinson MC, & Fernig DG (2016). Heparin binding preference and structures in the fibroblast growth factor family parallel their evolutionary diversification. *Open Biology*, 6(3), 150275–150275. doi:10.1098/rsob.150275 [PubMed: 27030175]
- Li Y, Zheng M, & Lau YF (2014). The sex-determining factors SRY and SOX9 regulate similar target genes and promote testis cord formation during testicular differentiation. *Cell Rep*, 8(3), 723–733. doi:10.1016/j.celrep.2014.06.055 [PubMed: 25088423]
- Lin CJ, Koh FM, Wong P, Conti M, & Ramalho-Santos M (2014). Hira-mediated H3.3 incorporation is required for DNA replication and ribosomal RNA transcription in the mouse zygote. *Dev Cell*, 30(3), 268–279. doi:10.1016/j.devcel.2014.06.022 [PubMed: 25087892]
- Lin Y, & Page DC (2005). Dazl deficiency leads to embryonic arrest of germ cell development in XY C57BL/6 mice. *Dev Biol*, 288(2), 309–316. doi:10.1016/j.ydbio.2005.06.032 [PubMed: 16310179]
- Lindeman RE, Gearhart MD, Minkina A, Krentz AD, Bardwell VJ, & Zarkower D (2015). Sexual cell-fate reprogramming in the ovary by DMRT1. *Curr Biol*, 25(6), 764–771. doi:10.1016/j.cub.2015.01.034 [PubMed: 25683803]
- Lintern-Moore S, & Moore GP (1979). The initiation of follicle and oocyte growth in the mouse ovary. *Biol Reprod*, 20(4), 773–778. [PubMed: 454765]
- Liu C, Peng J, Matzuk MM, & Yao HH (2015). Lineage specification of ovarian theca cells requires multicellular interactions via oocyte and granulosa cells. *Nat Commun*, 6, 6934. doi:10.1038/ncomms7934 [PubMed: 25917826]
- Liu S, Brind'Amour J, Karimi MM, Shirane K, Bogutz A, Lefebvre L, ... Lorincz MC (2014). Setdb1 is required for germline development and silencing of H3K9me3-marked endogenous

retroviruses in primordial germ cells. *Genes and Development*, 28, 2041–2055. doi:10.1101/gad.244848.114 [PubMed: 25228647]

Liu X, Wang C, Liu W, Li J, Li C, Kou X, ... Gao S (2016). Distinct features of H3K4me3 and H3K27me3 chromatin domains in pre-implantation embryos. *Nature*, 537, 558–562. doi:10.1038/nature19362 [PubMed: 27626379]

Lo KC, Lei Z, Rao Ch V, Beck J, & Lamb DJ (2004). De novo testosterone production in luteinizing hormone receptor knockout mice after transplantation of leydig stem cells. *Endocrinology*, 145(9), 4011–4015. doi:10.1210/en.2003-1729 [PubMed: 15123536]

Loebel DA, Watson CM, De Young RA, & Tam PP (2003). Lineage choice and differentiation in mouse embryos and embryonic stem cells. *Dev Biol*, 264(1), 1–14. [PubMed: 14623228]

Loppin B, Bonnefoy E, Anselme C, Laurencon A, Karr TL, & Couble P (2005). The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. *Nature*, 437(7063), 1386–1390. doi:10.1038/nature04059 [PubMed: 16251970]

Lovell-Badge R, & Robertson E (1990). XY female mice resulting from a heritable mutation in the primary testis-determining gene, Tdy. *Development*, 109(3), 635–646. [PubMed: 2401216]

Lucifero D, Mann MRW, Bartolomei MS, & Trasler JM (2004). Gene-specific timing and epigenetic memory in oocyte imprinting. *Human Molecular Genetics*, 13, 839–849. doi:10.1093/hmg/ddh104 [PubMed: 14998934]

Luo L, Chen H, Stocco DM, & Zirkin BR (1998). Leydig Cell Protein Synthesis and Steroidogenesis in Response to Acute Stimulation by Luteinizing Hormone in Rats 1. *Biology of Reproduction*, 59, 263–270. doi:10.1095/biolreprod59.2.263 [PubMed: 9687294]

Luo X, Ikeda Y, & Parker KL (1994). A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell*, 77(4), 481–490. [PubMed: 8187173]

Magnusdottir E, Dietmann S, Murakami K, Gunesdogan U, Tang F, Bao S, ... Azim Surani M (2013). A tripartite transcription factor network regulates primordial germ cell specification in mice. *Nat Cell Biol*, 15(8), 905–915. doi:10.1038/ncb2798 [PubMed: 23851488]

Malki S, van der Heijden GW, O'Donnell KA, Martin SL, & Bortvin A (2014). A role for retrotransposon LINE-1 in fetal oocyte attrition in mice. *Dev Cell*, 29(5), 521–533. doi:10.1016/j.devcel.2014.04.027 [PubMed: 24882376]

Marston AL, & Amon A (2004). Meiosis: Cell-cycle controls shuffle and deal. *Nature Reviews Molecular Cell Biology*, 5, 983–997. doi:10.1038/nrm1526 [PubMed: 15573136]

Martineau J, Nordqvist K, Tilmann C, Lovell-Badge R, & Capel B (1997). Male-specific cell migration into the developing gonad. *Curr Biol*, 7(12), 958–968. [PubMed: 9382843]

Martinovitch PN (1937). Development in vitro of the Mammalian Gonad. *Nature*, 139, 413. doi:10.1038/139413a0

Matova N, & Cooley L (2001). Comparative aspects of animal oogenesis. *Dev Biol*, 231(2), 291–320. doi:10.1006/dbio.2000.0120 [PubMed: 11237461]

Mayer W, Niveleau A, Walter J, Fundele R, & Haaf T (2000). Embryogenesis: Demethylation of the zygotic paternal genome. *Nature*, 403, 501–502. doi:10.1038/35000656 [PubMed: 10676950]

McLaren A (1984). Meiosis and differentiation of mouse germ cells. *Symp Soc Exp Biol*, 38, 7–23. [PubMed: 6400220]

McLaren A (2003). Primordial germ cells in the mouse. *Dev Biol*, 262(1), 1–15. [PubMed: 14512014]

Mcvean GAT, Myers SR, & Hunt S (2012). The Fine-Scale Structure of Recombination Rate Variation in the Human Genome. 581, 581–585. doi:10.1126/science.1092500

Meng J, Holdcraft RW, Shima JE, Griswold MD, & Braun RE (2005). Androgens regulate the permeability of the blood-testis barrier. *Proceedings of the National Academy of Sciences*, 102, 16696–16700. doi:10.1073/pnas.0506084102

Meng X, Lindahl M, Hyvonen ME, Parvinen M, de Rooij DG, Hess MW, ... Sariola H (2000). Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. *Science*, 287(5457), 1489–1493. [PubMed: 10688798]

Menke DB, Koubova J, & Page DC (2003). Sexual differentiation of germ cells in XX mouse gonads occurs in an anterior-to-posterior wave. *Dev Biol*, 262(2), 303–312. [PubMed: 14550793]

- Miki H, Lee J, Inoue K, Ogonuki N, Noguchi Y, Mochida K, ... Ogura A (2004). Microinsemination with first-wave round spermatids from immature male mice. *The Journal of reproduction and development*, 50, 131–137. doi:10.1262/jrd.50.131 [PubMed: 15007210]
- Miyamoto N, Yoshida M, Kuratani S, Matsuo I, & Aizawa S (1997). Defects of urogenital development in mice lacking *Emx2*. *Development*, 124(9), 1653–1664. [PubMed: 9165114]
- Molyneaux KA, Stallock J, Schaible K, & Wylie C (2001). Time-Lapse Analysis of Living Mouse Germ Cell Migration. *Developmental Biology*, 240(2), 488–498. doi:10.1006/dbio.2001.0436 [PubMed: 11784078]
- Molyneaux KA, Zinszner H, Kunwar PS, Schaible K, Stebler J, Sunshine MJ, ... Lehmann R (2003). The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. *Development*, 130(18), 4279 LP–4286. [PubMed: 12900445]
- Moore GP, & Lintern-Moore S (1979). Stimulation of endogenous RNA polymerase I activity in the mouse oocyte after PMSG treatment. *Biol Reprod*, 21(2), 373–377. [PubMed: 486661]
- Morais da Silva S, Hacker A, Harley V, Goodfellow P, Swain A, & Lovell-Badge R (1996). Sox9 expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. *Nat Genet*, 14(1), 62–68. doi:10.1038/ng0996-62 [PubMed: 8782821]
- Morales A, Mohamed F, & Cavicchia JC (2007). Apoptosis and blood-testis barrier during the first spermatogenic wave in the pubertal rat. *Anatomical Record*, 290, 206–214. doi:10.1002/ar.20417
- Moreno RD, Lizama C, Urzúa N, Vergara SP, & Reyes JG (2006). Caspase activation throughout the first wave of spermatogenesis in the rat. *Cell and Tissue Research*, 325, 533–540. doi:10.1007/s00441-006-0186-4 [PubMed: 16598500]
- Morgan M, Much C, DiGiacomo M, Azzi C, Ivanova I, Vitsios DM, ... O'Carroll D (2017). mRNA 3' uridylation and poly(A) tail length sculpt the mammalian maternal transcriptome. *Nature*, 548(7667), 347–351. doi:10.1038/nature23318 [PubMed: 28792939]
- Mori C, Nakamura N, Dix DJ, Fujioka M, Nakagawa S, Shiota K, & Eddy EM (1997). Morphological analysis of germ cell apoptosis during postnatal testis development in normal and Hsp 70–2 knockout mice. *Developmental dynamics* : an official publication of the American Association of Anatomists, 208, 125–136. doi:10.1002/(SICI)1097-0177(199701)208:1<125::AID-AJA12>3.0.CO;2-5 [PubMed: 8989527]
- Mork L, Maatouk DM, McMahon JA, Guo JJ, Zhang P, McMahon AP, & Capel B (2012). Temporal Differences in Granulosa Cell Specification in the Ovary Reflect Distinct Follicle Fates in Mice. *Biology of Reproduction*, 86(2), 1–9, 1–37, 37.
- Morohaku K, Tanimoto R, Sasaki K, Kawahara-Miki R, Kono T, Hayashi K, ... Obata Y (2016). Complete in vitro generation of fertile oocytes from mouse primordial germ cells. *Proc Natl Acad Sci U S A*, 113(32), 9021–9026. doi:10.1073/pnas.1603817113 [PubMed: 27457928]
- Myers S (2005). A Fine-Scale Map of Recombination Rates and Hotspots Across the Human Genome. *Science*, 310, 321–324. doi:10.1126/science.1117196 [PubMed: 16224025]
- Myers S, Freeman C, Auton A, Donnelly P, & McVean G (2008). A common sequence motif associated with recombination hot spots and genome instability in humans. *Nature Genetics*, 40, 1124–1129. doi:10.1038/ng.213 [PubMed: 19165926]
- Nagamatsu G, & Hayashi K (2017). Stem cells, in vitro gametogenesis and male fertility. *Reproduction*, 154(6), F79–F91. doi:10.1530/REP-17-0510 [PubMed: 29133304]
- Nakagawa T, Sharma M, Nabeshima Y, Braun RE, & Yoshida S (2010). Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. *Science*, 328(5974), 62–67. doi:10.1126/science.1182868 [PubMed: 20299552]
- Nakagawa T, Sharma M, Nabeshima Y. i., Braun RE, Yoshida Shosei. (2010). Functional Hierarchy and Reversibility within the Murine Spermatogenic Stem Cell Compartment. 328, 62–67. doi:10.1126/science.1182868.Functional
- Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, ... Nakano T (2007). PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nature Cell Biology*, 9, 64–71. doi:10.1038/ncb1519 [PubMed: 17143267]

- Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, Matoba S, ... Nakano T (2012). PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature*, 486, 415–419. doi:10.1038/nature11093 [PubMed: 22722204]
- Namwanje M, & Brown CW (2016). Activins and Inhibins: Roles in Development, Physiology, and Disease. *Cold Spring Harb Perspect Biol*, 8(7). doi:10.1101/cshperspect.a021881
- Naughton CK, Jain S, Strickland AM, Gupta A, & Milbrandt J (2006). Glial cell-line derived neurotrophic factor-mediated RET signaling regulates spermatogonial stem cell fate. *Biol Reprod*, 74(2), 314–321. doi:10.1095/biolreprod.105.047365 [PubMed: 16237148]
- Naughton CK, Jain S, Strickland AM, Gupta A, & Milbrandt J (2006). Glial Cell-Line Derived Neurotrophic Factor-Mediated RET Signaling Regulates Spermatogonial Stem Cell Fate1. *Biology of Reproduction*, 74(2), 314–321. [PubMed: 16237148]
- Nayernia K, Nolte J, Michelmann HW, Lee JH, Rathack K, Drusenheimer N, ... Engel W (2006). In vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. *Dev Cell*, 11(1), 125–132. doi:10.1016/j.devcel.2006.05.010 [PubMed: 16824959]
- Nef S, Verma-Kurvari S, Merenmies J, Vassalli JD, Efstratiadis A, Accili D, & Parada LF (2003). Testis determination requires insulin receptor family function in mice. *Nature*, 426(6964), 291–295. doi:10.1038/nature02059 [PubMed: 14628051]
- Ng JH, Kumar V, Muratani M, Kraus P, Yeo JC, Yaw LP, ... Ng HH (2013). In Vivo Epigenomic Profiling of Germ Cells Reveals Germ Cell Molecular Signatures. *Developmental Cell*, 24, 324–333. doi:10.1016/j.devcel.2012.12.011 [PubMed: 23352811]
- Ng JH, Kumar V, Muratani M, Kraus P, Yeo JC, Yaw LP, ... Ng HH (2013). In vivo epigenomic profiling of germ cells reveals germ cell molecular signatures. *Dev Cell*, 24(3), 324–333. doi:10.1016/j.devcel.2012.12.011 [PubMed: 23352811]
- Nicol B, & Yao HH (2014). Building an ovary: insights into establishment of somatic cell lineages in the mouse. *Sex Dev*, 8(5), 243–251. doi:10.1159/000358072 [PubMed: 24480990]
- O'Shaughnessy PJ, Monteiro A, Verhoeven G, De Gendt K, & Abel MH (2010). Effect of FSH on testicular morphology and spermatogenesis in gonadotrophin-deficient hypogonadal mice lacking androgen receptors. *Reproduction*, 139, 177–184. doi:10.1530/REP-09-0377 [PubMed: 19846485]
- Oakberg EF (1968). Mammalian gametogenesis and species comparisons in radiation response of the gonads In *Effects of radiation on meiotic systems. Report of a study group* (pp. 3–15). Vienna: Vienna: International Atomic Energy Agency.
- Oakberg EF (1971). Spermatogonial stem-cell renewal in the mouse. *Anat Rec*, 169(3), 515–531. doi:10.1002/ar.1091690305 [PubMed: 5550531]
- Oatley JM, Avarbock MR, & Brinster RL (2007). Glial cell line-derived neurotrophic factor regulation of genes essential for self-renewal of mouse spermatogonial stem cells is dependent on Src family kinase signaling. *Journal of Biological Chemistry*, 282, 25842–25851. doi:10.1074/jbc.M703474200 [PubMed: 17597063]
- Oatley JM, Avarbock MR, Telaranta AI, Fearon DT, & Brinster RL (2006). Identifying genes important for spermatogonial stem cell self-renewal and survival. *Proceedings of the National Academy of Sciences*, 103(25), 9524 LP–9529.
- Oatley MJ, Kaucher AV, Racicot KE, & Oatley JM (2011). Inhibitor of DNA binding 4 is expressed selectively by single spermatogonia in the male germline and regulates the self-renewal of spermatogonial stem cells in mice. *Biol Reprod*, 85(2), 347–356. doi:10.1095/biolreprod.111.091330 [PubMed: 21543770]
- Oatley MJ, Kaucher AV, Racicot KE, & Oatley JM (2011). Inhibitor of DNA Binding 4 Is Expressed Selectively by Single Spermatogonia in the Male Germline and Regulates the Self-Renewal of Spermatogonial Stem Cells in Mice1. *Biology of Reproduction*, 85, 347–356. doi:10.1095/biolreprod.111.091330 [PubMed: 21543770]
- Obata Y, Kono T, & Hatada I (2002). Gene silencing: maturation of mouse fetal germ cells in vitro. *Nature*, 418(6897), 497. doi:10.1038/418497a [PubMed: 12152066]
- Oh B, Hwang S, McLaughlin J, Solter D, & Knowles BB (2000). Timely translation during the mouse oocyte-to-embryo transition. *Development*, 127(17), 3795–3803. [PubMed: 10934024]

- Ohinata Y, Ohta H, Shigeta M, Yamanaka K, Wakayama T, & Saitou M (2009). A signaling principle for the specification of the germ cell lineage in mice. *Cell*, 137(3), 571–584. doi:10.1016/j.cell.2009.03.014 [PubMed: 19410550]
- Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, ... Surani MA (2005). Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature*, 436, 207–213. doi:10.1038/nature03813 [PubMed: 15937476]
- Ohta H, Tohda A, & Nishimune Y (2003). Proliferation and differentiation of spermatogonial stem cells in the w/wv mutant mouse testis. *Biol Reprod*, 69(6), 1815–1821. doi:10.1095/biolreprod.103.019323 [PubMed: 12890724]
- Oktyay K, Briggs D, & Gosden RG (1997). Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. *J Clin Endocrinol Metab*, 82(11), 3748–3751. doi:10.1210/jcem.82.11.4346 [PubMed: 9360535]
- Oktyay K, Newton H, Mullan J, & Gosden RG (1998). Development of human primordial follicles to antral stages in SCID/hpg mice stimulated with follicle stimulating hormone. *Hum Reprod*, 13(5), 1133–1138. [PubMed: 9647533]
- Oswald J, Engemann S, Lane N, Mayer W, Olek A, Fundele R, ... Walter J (2000). Active demethylation of the paternal genome in the mouse zygote. *Current Biology*, 10, 475–478. doi:10.1016/S0960-9822(00)00448-6 [PubMed: 10801417]
- Pailhoux E, Vigier B, Chaffaux S, Servel N, Taourit S, Furet JP, ... Vaiman D (2001). A 11.7-kb deletion triggers intersexuality and polledness in goats. *Nat Genet*, 29(4), 453–458. doi:10.1038/ng769 [PubMed: 11726932]
- Pailhoux E, Vigier B, Vaiman D, Servel N, Chaffaux S, Cribiu EP, & Cotinot C (2002). Ontogenesis of female-to-male sex-reversal in XX polled goats. *Dev Dyn*, 224(1), 39–50. doi:10.1002/dvdy.10083 [PubMed: 11984872]
- Palmer SJ, & Burgoyne PS (1991). In situ analysis of fetal, prepuberal and adult XX----XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. *Development*, 112(1), 265–268. [PubMed: 1769333]
- Pangas SA, Choi Y, Ballow DJ, Zhao Y, Westphal H, Matzuk MM, & Rajkovic A (2006). Oogenesis requires germ cell-specific transcriptional regulators *Sohlh1* and *Lhx8*. *Proc Natl Acad Sci U S A*, 103(21), 8090–8095. doi:10.1073/pnas.0601083103 [PubMed: 16690745]
- Pangas SA, Jorgez CJ, Tran M, Agno J, Li X, Brown CW, ... Matzuk MM (2007). Intraovarian activins are required for female fertility. *Mol Endocrinol*, 21(10), 2458–2471. doi:10.1210/me.2007-0146 [PubMed: 17609433]
- Paniagua R, Codesal J, Nistal M, Rodriguez MC, & Santamaria L (1987). Quantification of cell types throughout the cycle of the human seminiferous epithelium and their DNA content. A new approach to the spermatogonial stem cell in man. *Anat Embryol (Berl)*, 176(2), 225–230. [PubMed: 3619074]
- Parma P, Radi O, Vidal V, Chaboissier MC, Dellambra E, Valentini S, ... Camerino G (2006). *R-spondin1* is essential in sex determination, skin differentiation and malignancy. *Nat Genet*, 38(11), 1304–1309. doi:10.1038/ng1907 [PubMed: 17041600]
- Parvanov ED, Petkov PM, & Paigen K (2010). *Prdm9* controls activation of mammalian recombination hotspots. *Science*, 327, 835. doi:10.1126/science.1181495 [PubMed: 20044538]
- Pastor WA, Liu W, Chen D, Ho J, Kim R, Hunt TJ, ... Clark AT (2018). TFAP2C regulates transcription in human naive pluripotency by opening enhancers. *Nat Cell Biol*, 20(5), 553–564. doi:10.1038/s41556-018-0089-0 [PubMed: 29695788]
- Pepling ME (2006). From primordial germ cell to primordial follicle: mammalian female germ cell development. *Genesis*, 44(12), 622–632. doi:10.1002/dvg.20258 [PubMed: 17146778]
- Pepling ME (2012). Follicular assembly: mechanisms of action. *Reproduction*, 143(2), 139–149. doi:10.1530/REP-11-0299 [PubMed: 22065859]
- Pepling ME, & Spradling AC (1998). Female mouse germ cells form synchronously dividing cysts. *Development*, 125(17), 3323 LP–3328. [PubMed: 9693136]
- Perez GI, Robles R, Knudson CM, Flaws JA, Korsmeyer SJ, & Tilly JL (1999). Prolongation of ovarian lifespan into advanced chronological age by *Bax*-deficiency. *Nat Genet*, 21(2), 200–203. doi:10.1038/5985 [PubMed: 9988273]

- Phillips BT, Gassei K, & Orwig KE (2010). Spermatogonial stem cell regulation and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci*, 365(1546), 1663–1678. doi:10.1098/rstb.2010.0026 [PubMed: 20403877]
- Pittman DL, Cobb J, Schimenti KJ, Wilson LA, Cooper DM, Brignull E, ... Schimenti JC (1998). Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. *Mol Cell*, 1(5), 697–705. [PubMed: 9660953]
- Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, ... Reik W (2010). Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. *Nature*, 463, 1101–1105. doi:10.1038/nature08829 [PubMed: 20098412]
- Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K. i., Brykczynska U, ... Peters AHFM (2008). PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nature Genetics*, 40, 411–420. doi:10.1038/ng.99 [PubMed: 18311137]
- Rajkovic A, Pangas SA, Ballow D, Suzumori N, & Matzuk MM (2004). NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science*, 305(5687), 1157–1159. doi:10.1126/science.1099755 [PubMed: 15326356]
- Rando OJ, & Rondo. (2016). Intergenerational Transfer of Epigenetic Information in Sperm. *Cold Spring Harbor Perspectives in Medicine*, 6, 1–14. doi:10.1101/cshperspect.a022988
- Ratts VS, Flaws JA, Kolp R, Sorenson CM, & Tilly JL (1995). Ablation of bcl-2 gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. *Endocrinology*, 136(8), 3665–3668. doi:10.1210/endo.136.8.7628407 [PubMed: 7628407]
- Raverdeau M, Gely-Pernot A, Feret B, Dennefeld C, Benoit G, Davidson I, ... Ghyselinck NB (2012). Retinoic acid induces Sertoli cell paracrine signals for spermatogonia differentiation but cell autonomously drives spermatocyte meiosis. *Proceedings of the National Academy of Sciences*, 109, 16582–16587. doi:10.1073/pnas.1214936109
- Raz E (2004). Guidance of primordial germ cell migration. *Curr Opin Cell Biol*, 16(2), 169–173. doi:10.1016/j.ceb.2004.01.004 [PubMed: 15196560]
- Raz E (2004). Guidance of primordial germ cell migration. *Current Opinion in Cell Biology*, 16(2), 169–173. doi:10.1016/j.ceb.2004.01.004 [PubMed: 15196560]
- Reddy P, Liu L, Adhikari D, Jagarlamudi K, Rajareddy S, Shen Y, ... Liu K (2008). Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. *Science*, 319(5863), 611–613. doi:10.1126/science.1152257 [PubMed: 18239123]
- Reith AD, Rottapel R, Giddens E, Brady C, Forrester L, & Bernstein A (1990). W mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-kit receptor. *Genes Dev*, 4(3), 390–400. [PubMed: 1692559]
- Ren Y, Suzuki H, Jagarlamudi K, Golnoski K, McGuire M, Lopes R, ... Rajkovic A (2015). Lhx8 regulates primordial follicle activation and postnatal folliculogenesis. *BMC Biol*, 13, 39. doi:10.1186/s12915-015-0151-3 [PubMed: 26076587]
- Richardson BE, & Lehmann R (2010). Mechanisms guiding primordial germ cell migration: Strategies from different organisms. *Nature Reviews Molecular Cell Biology*, 11(1), 37–49. doi:10.1038/nrm2815 [PubMed: 20027186]
- Romanienko PJ, & Camerini-Otero RD (2000). The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol Cell*, 6(5), 975–987.
- Romanienko PJ, & Camerini-Otero RD (2000). The mouse Spo11 gene is required for meiotic chromosome synapsis. *Molecular Cell*, 6, 975–987. doi:10.1016/S1097-2765(00)00097-6
- Ross AJ, Tilman C, Yao H, MacLaughlin D, & Capel B (2003). AMH induces mesonephric cell migration in XX gonads. *Mol Cell Endocrinol*, 211(1–2), 1–7.
- Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, ... Cooke HJ (1997). The mouse Dazl gene encodes a cytoplasmic protein essential for gametogenesis. *Nature*, 389(6646), 73–77. doi:10.1038/37987 [PubMed: 9288969]
- Runyan C, Schaible K, Molyneaux K, Wang Z, Levin L, & Wylie C (2006). Steel factor controls midline cell death of primordial germ cells and is essential for their normal proliferation and migration. *Development*, 133(24), 4861–4869. doi:10.1242/dev.02688 [PubMed: 17107997]

- Russell LD, Chiarini-Garcia H, Korsmeyer SJ, & Knudson CM (2002). Bax-dependent spermatogonia apoptosis is required for testicular development and spermatogenesis. *Biol Reprod*, 66(4), 950–958. [PubMed: 11906913]
- Russell LD, E. R, SinhaHikim AP, Clegg ED. (1990). *Histological and Histopathological Evaluation of the Testis*. Clearwater, FL: Cache River Press.
- Ruwanpura SM, McLachlan RI, Matthiesson KL, & Meachem SJ (2008). Gonadotrophins regulate germ cell survival, not proliferation, in normal adult men. *Hum Reprod*, 23(2), 403–411. doi:10.1093/humrep/dem376 [PubMed: 18199736]
- Saitou M, Barton SC, & Surani MA (2002). A molecular programme for the specification of germ cell fate in mice. *Nature*, 418(6895), 293–300. doi:10.1038/nature00927 [PubMed: 12124616]
- Saitou M, & Yamaji M (2012). Primordial germ cells in mice. *Cold Spring Harb Perspect Biol*, 4(11). doi:10.1101/cshperspect.a008375
- Santenard A, Ziegler-Birling C, Koch M, Tora L, Bannister AJ, & Torres-Padilla ME (2010). Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. *Nat Cell Biol*, 12(9), 853–862. doi:10.1038/ncb2089 [PubMed: 20676102]
- Sasaki K, Yokobayashi S, Nakamura T, Okamoto I, Yabuta Y, Kurimoto K, ... Saitou M (2015). Robust In Vitro Induction of Human Germ Cell Fate from Pluripotent Stem Cells. *Cell Stem Cell*, 17(2), 178–194. doi:10.1016/j.stem.2015.06.014 [PubMed: 26189426]
- Sato M, Kimura T, Kurokawa K, Fujita Y, Abe K, Masuhara M, ... Nakano T (2002). Identification of PGC7, a new gene expressed specifically in preimplantation embryos and germ cells. *Mechanisms of Development*, 113(1), 91–94. doi:10.1016/S0925-4773(02)00002-3 [PubMed: 11900980]
- Saunders PT, Turner JM, Ruggiu M, Taggart M, Burgoyne PS, Elliott D, & Cooke HJ (2003). Absence of mDazl produces a final block on germ cell development at meiosis. *Reproduction*, 126(5), 589–597. [PubMed: 14611631]
- Schlatt S, & Weinbauer GF (1994). Immunohistochemical localization of proliferating cell nuclear antigen as a tool to study cell proliferation in rodent and primate testes. *Int J Androl*, 17(4), 214–222. [PubMed: 7995658]
- Schmahl J, Kim Y, Colvin JS, Ornitz DM, & Capel B (2004). Fgf9 induces proliferation and nuclear localization of FGFR2 in Sertoli precursors during male sex determination. *Development*, 131(15), 3627–3636. doi:10.1242/dev.01239 [PubMed: 15229180]
- Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier AC, & Treier M (2004). The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. *Development*, 131(4), 933–942. doi:10.1242/dev.00969 [PubMed: 14736745]
- Schöler HR, Hatzopoulos AK, Balling R, Suzuki N, & Gruss P (1989). A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. *The EMBO Journal*, 8(9), 2543–2550. [PubMed: 2573523]
- Schöler HR, Ruppert S, Suzuki N, Chowdhury K, & Gruss P (1990). New type of POU domain in germ line-specific protein Oct-4. *Nature*, 344, 435–435. [PubMed: 1690859]
- Schrans-Stassen BH, Saunders PT, Cooke HJ, & de Rooij DG (2001). Nature of the spermatogenic arrest in Dazl $-/-$ mice. *Biol Reprod*, 65(3), 771–776. [PubMed: 11514340]
- Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, ... Reik W (2012). The Dynamics of Genome-wide DNA Methylation Reprogramming in Mouse Primordial Germ Cells. *Molecular Cell*, 48, 849–862. doi:10.1016/j.molcel.2012.11.001 [PubMed: 23219530]
- Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, ... Reik W (2012). The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol Cell*, 48(6), 849–862. doi:10.1016/j.molcel.2012.11.001 [PubMed: 23219530]
- Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M, & Matsui Y (2005). Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. *Developmental Biology*, 278, 440–458. doi:10.1016/j.ydbio.2004.11.025 [PubMed: 15680362]
- Seki Y, Yamaji M, Yabuta Y, Sano M, Shigeta M, Matsui Y, ... Saitou M (2007). Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. *Development*, 134, 2627–2638. doi:10.1242/dev.005611 [PubMed: 17567665]

- Seligman J, & Page DC (1998). The *Dazl* gene is expressed in male and female embryonic gonads before germ cell sex differentiation. *Biochem Biophys Res Commun*, 245(3), 878–882. doi:10.1006/bbrc.1998.8530 [PubMed: 9588208]
- Shirane K, Kurimoto K, Yabuta Y, Yamaji M, Satoh J, Ito S, ... Sasaki H (2016). Global Landscape and Regulatory Principles of DNA Methylation Reprogramming for Germ Cell Specification by Mouse Pluripotent Stem Cells. *Developmental Cell*, 39, 87–103. doi:10.1016/j.devcel.2016.08.008 [PubMed: 27642137]
- Shuhaibar LC, Egbert JR, Norris RP, Lampe PD, Nikolaev VO, Thunemann M, ... Jaffe LA (2015). Intercellular signaling via cyclic GMP diffusion through gap junctions restarts meiosis in mouse ovarian follicles. *Proc Natl Acad Sci U S A*, 112(17), 5527–5532. doi:10.1073/pnas.1423598112 [PubMed: 25775542]
- Siklenka K, Erkek S, Godmann M, Lambrot R, McGraw S, Lafleur C, ... Kimmins S (2015). Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science*, 350(6261), aab2006. doi:10.1126/science.aab2006 [PubMed: 26449473]
- Simorangkir DR, Marshall GR, & Plant TM (2009). A re-examination of proliferation and differentiation of type A spermatogonia in the adult rhesus monkey (*Macaca mulatta*). *Hum Reprod*, 24(7), 1596–1604. doi:10.1093/humrep/dep051 [PubMed: 19282325]
- Smallwood SA, Tomizawa SI, Krueger F, Ruf N, Carli N, Segonds-Pichon A, ... Kelsey G (2011). Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nature Genetics*, 43, 811–814. doi:10.1038/ng.864 [PubMed: 21706000]
- Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, & Meissner A (2012). A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature*, 484, 339–344. doi:10.1038/nature10960 [PubMed: 22456710]
- Smith ZD, & Meissner A (2013). DNA methylation: Roles in mammalian development. *Nature Reviews Genetics*, 14, 204–220. doi:10.1038/nrg3354
- Sollier J, Lin W, Soustelle C, Suhre K, Nicolas A, Geli V, & de La Roche Saint-Andre C (2004). *Set1* is required for meiotic S-phase onset, double-strand break formation and middle gene expression. *EMBO J*, 23(9), 1957–1967. doi:10.1038/sj.emboj.7600204 [PubMed: 15071505]
- Stewart KR, Veselovska L, & Kelsey G (2016). Establishment and functions of DNA methylation in the germline. *Epigenomics*, 8, 1399–1413. doi:10.2217/epi-2016-0056 [PubMed: 27659720]
- Stewart KR, Veselovska L, Kim J, Huang J, Saadeh H, Tomizawa SI, ... Kelsey G (2015). Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. *Genes and Development*, 29, 2449–2462. doi:10.1101/gad.271353.115 [PubMed: 26584620]
- Suzuki A, Sugihara A, Uchida K, Sato T, Ohta Y, Katsu Y, ... Iguchi T (2002). Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. *Reprod Toxicol*, 16(2), 107–116. [PubMed: 11955941]
- Suzuki H, Sada A, Yoshida S, & Saga Y (2009). The heterogeneity of spermatogonia is revealed by their topology and expression of marker proteins including the germ cell-specific proteins *Nanos2* and *Nanos3*. *Dev Biol*, 336(2), 222–231. doi:10.1016/j.ydbio.2009.10.002 [PubMed: 19818747]
- Svoboda P, Franke V, & Schultz RM (2015). Sculpting the Transcriptome During the Oocyte-to-Embryo Transition in Mouse. *Curr Top Dev Biol*, 113, 305–349. doi:10.1016/bs.ctdb.2015.06.004 [PubMed: 26358877]
- Swain A, Narvaez V, Burgoyne P, Camerino G, & Lovell-Badge R (1998). *Dax1* antagonizes *Sry* action in mammalian sex determination. *Nature*, 391(6669), 761–767. doi:10.1038/35799 [PubMed: 9486644]
- Takashima S, Kanatsu-Shinohara M, Tanaka T, Morimoto H, Inoue K, Ogonuki N, ... Shinohara T (2015). Functional Differences between GDNF-Dependent and FGF2-Dependent Mouse Spermatogonial Stem Cell Self-Renewal. *Stem Cell Reports*, 4(3), 489–502. doi:10.1016/j.stemcr.2015.01.010 [PubMed: 25684228]
- Tam PPL, & Zhou SX (1996). The allocation of epiblast cells to ectodermal and germ-line lineages is influenced by the position of the cells in the gastrulating mouse embryo. *Developmental Biology*, 178(1), 124–132. doi:10.1006/dbio.1996.0203 [PubMed: 8812114]

- Tang WWC, Dietmann S, Irie N, Leitch HG, Floros VI, Bradshaw CR, ... Surani MA (2015). A unique gene regulatory network resets the human germline epigenome for development. *Cell*, 161, 1453–1467. doi:10.1016/j.cell.2015.04.053 [PubMed: 26046444]
- Tang WWC, Kobayashi T, Irie N, Dietmann S, & Surani MA (2016). Specification and epigenetic programming of the human germ line. *Nature Reviews Genetics*, 17, 585–600. doi:10.1038/nrg.2016.88
- Tardat M, Albert M, Kunzmann R, Liu Z, Kaustov L, Thierry R, ... Peters AH (2015). Cbx2 targets PRC1 to constitutive heterochromatin in mouse zygotes in a parent-of-origin-dependent manner. *Mol Cell*, 58(1), 157–171. doi:10.1016/j.molcel.2015.02.013 [PubMed: 25801166]
- Teixeira Filho FL, Baracat EC, Lee TH, Suh CS, Matsui M, Chang RJ, ... Erickson GF (2002). Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. *J Clin Endocrinol Metab*, 87(3), 1337–1344. doi:10.1210/jcem.87.3.8316 [PubMed: 11889206]
- Tokuda M, Kadokawa Y, Kurahashi H, & Marunouchi T (2007). CDH1 is a specific marker for undifferentiated spermatogonia in mouse testes. *Biol Reprod*, 76(1), 130–141. doi:10.1095/biolreprod.106.053181 [PubMed: 17035642]
- Tomizuka K, Horikoshi K, Kitada R, Sugawara Y, Iba Y, Kojima A, ... Kakitani M (2008). R-spondin1 plays an essential role in ovarian development through positively regulating Wnt-4 signaling. *Hum Mol Genet*, 17(9), 1278–1291. doi:10.1093/hmg/ddn036 [PubMed: 18250097]
- Torres-Padilla ME, Bannister AJ, Hurd PJ, Kouzarides T, & Zernicka-Goetz M (2006). Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos. *Int J Dev Biol*, 50(5), 455–461. doi:10.1387/ijdb.052073mt [PubMed: 16586346]
- Tremblay KD, Dunn NR, & Robertson EJ (2001). Mouse embryos lacking Smad1 signals display defects in extra-embryonic tissues and germ cell formation. *Development*, 128(18), 3609–3621. [PubMed: 11566864]
- Tsai M-Y, Yeh S-D, Wang R-S, Yeh S, Zhang C, Lin H-Y, ... Chang C (2006). Differential effects of spermatogenesis and fertility in mice lacking androgen receptor in individual testis cells. *Proceedings of the National Academy of Sciences*, 103, 18975–18980. doi:10.1073/pnas.0608565103
- Uda M, Ottolenghi C, Crisponi L, Garcia JE, Deiana M, Kimber W, ... Pilia G (2004). Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. *Hum Mol Genet*, 13(11), 1171–1181. doi:10.1093/hmg/ddh124 [PubMed: 15056605]
- Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, ... Treier M (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell*, 139(6), 1130–1142. doi:10.1016/j.cell.2009.11.021 [PubMed: 20005806]
- Vainio S, Heikkilä M, Kispert A, Chin N, & McMahon AP (1999). Female development in mammals is regulated by Wnt-4 signalling. *Nature*, 397(6718), 405–409. doi:10.1038/17068 [PubMed: 9989404]
- Valli H, Sukhwani M, Dovey SL, Peters KA, Donohue J, Castro CA, ... Orwig KE (2014). Fluorescence- and magnetic-activated cell sorting strategies to isolate and enrich human spermatogonial stem cells. *Fertil Steril*, 102(2), 566–580 e567. doi:10.1016/j.fertnstert.2014.04.036 [PubMed: 24890267]
- van Alphen MM, van de Kant HJ, & de Rooij DG (1988). Depletion of the spermatogonia from the seminiferous epithelium of the rhesus monkey after X irradiation. *Radiat Res*, 113(3), 473–486. [PubMed: 3347704]
- van Bragt MP, Roepers-Gajadien HL, Korver CM, Bogerd J, Okuda A, Eggen BJ, ... van Pelt AM (2008). Expression of the pluripotency marker UTF1 is restricted to a subpopulation of early A spermatogonia in rat testis. *Reproduction*, 136(1), 33–40. doi:10.1530/REP-07-0536 [PubMed: 18390688]
- Van Der Heijden GW, Derijck AAHA, Pósfai E, Giele M, Pelczar P, Ramos L, ... De Boer P (2007). Chromosome-wide nucleosome replacement and H3.3 incorporation during mammalian meiotic sex chromosome inactivation. *Nature Genetics*, 39, 251–258. doi:10.1038/ng1949 [PubMed: 17237782]

- Van Pelt AMM, & De Rooij DG (1990a). The Origin of the Synchronization of the Seminiferous Epithelium in Vitamin A-Deficient Rats after Vitamin A Replacement. *Biology of Reproduction*, 42, 677–682. [PubMed: 2346774]
- Van Pelt AMM, & de Rooij DG (1990b). Synchronization of the Seminiferous Epithelium after Vitamin A Replacement in Vitamin A-Deficient Mice. *Biology of Reproduction*, 43, 363–367. [PubMed: 2271719]
- Van Pelt AMM, & De Rooij DG (1991). Retinoic Acid Is Able to Reinitiate Spermatogenesis in Vitamin A-Deficient Rats and High Replicate Doses Support the Full Development of Spermatogenic Cells. *Endocrinology*, 128, 697–704. [PubMed: 1989855]
- Vidal VP, Chaboissier MC, de Rooij DG, & Schedl A (2001). Sox9 induces testis development in XX transgenic mice. *Nat Genet*, 28(3), 216–217. doi:10.1038/90046 [PubMed: 11431689]
- Vincent JJ, Huang Y, Chen PY, Feng S, Calvopiña JH, Nee K, ... Clark AT (2013). Stage-specific roles for Tet1 and Tet2 in DNA demethylation in primordial germ cells. *Cell Stem Cell*, 12, 470–478. doi:10.1016/j.stem.2013.01.016 [PubMed: 23415914]
- Vincent SD, Dunn NR, Sciammas R, Shapiro-Shalef M, Davis MM, Calame K, ... Robertson EJ (2005). The zinc finger transcriptional repressor Blimp1/Prdm1 is dispensable for early axis formation but is required for specification of primordial germ cells in the mouse. *Development*, 132(6), 1315–1325. doi:10.1242/dev.01711 [PubMed: 15750184]
- von Kopylow K, & Spiess AN (2017). Human spermatogonial markers. *Stem Cell Res*, 25, 300–309. doi:10.1016/j.scr.2017.11.011 [PubMed: 29239848]
- Weber S, Eckert D, Nettersheim D, Gillis AJM, Schäfer S, Kuckenberg P, ... Schorle H (2010). Critical Function of AP-2gamma/TCFAP2C in Mouse Embryonic Germ Cell Maintenance1. *Biology of Reproduction*, 82, 214–223. doi:10.1095/biolreprod.109.078717 [PubMed: 19776388]
- Western PS, Miles DC, van den Bergen JA, Burton M, & Sinclair AH (2008). Dynamic regulation of mitotic arrest in fetal male germ cells. *Stem Cells*, 26(2), 339–347. doi:10.1634/stemcells.2007-0622 [PubMed: 18024419]
- Wilhelm D, Palmer S, & Koopman P (2007). Sex determination and gonadal development in mammals. *Physiol Rev*, 87(1), 1–28. doi:10.1152/physrev.00009.2006 [PubMed: 17237341]
- Willems A, Batlouni SR, Esnal A, Swinnen JV, Saunders PTK, Sharpe RM, ... Verhoeven G (2010). Selective ablation of the androgen receptor in mouse sertoli cells affects sertoli cell maturation, barrier formation and cytoskeletal development. *PLoS One*, 5. doi:10.1371/journal.pone.0014168
- Willerton L, Smith RA, Russell D, & Mackay S (2004). Effects of FGF9 on embryonic Sertoli cell proliferation and testicular cord formation in the mouse. *Int J Dev Biol*, 48(7), 637–643. doi:10.1387/ijdb.031778lw [PubMed: 15470636]
- Williams CJ, & Erickson GF (2000). Morphology and Physiology of the Ovary In De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, & Vinik A (Eds.), *Endotext*. South Dartmouth (MA).
- Wu BJ, Dong FL, Ma XS, Wang XG, Lin F, & Liu HL (2014). Localization and expression of histone H2A variants during mouse oogenesis and preimplantation embryo development. *Genetics and Molecular Research*, 13, 5929–5939. doi:10.4238/2014.August.7.8 [PubMed: 25117351]
- Wu SF, Zhang H, & Cairns BR (2011). Genes for embryo development are packaged in blocks of multivalent chromatin in zebrafish sperm. *Genome Res*, 21(4), 578–589. doi:gr.113167.110 [pii] 10.1101/gr.113167.110 [PubMed: 21383318]
- Yamada T, Mizuno K, Hirota K, Kon N, Wahls WP, Hartsuiker E, ... Ohta K (2004). Roles of histone acetylation and chromatin remodeling factor in a meiotic recombination hotspot. *EMBO J*, 23(8), 1792–1803. doi:10.1038/sj.emboj.7600138 [PubMed: 14988732]
- Yamaguchi S, Hong K, Liu R, Inoue A, Shen L, Zhang K, & Zhang Y (2013). Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. *Cell Research*, 23, 329–339. doi:10.1038/cr.2013.22 [PubMed: 23399596]
- Yamaguchi S, Hong K, Liu R, Shen L, Inoue A, Diep D, ... Zhang Y (2012). Tet1 controls meiosis by regulating meiotic gene expression. *Nature*, 492, 443–447. doi:10.1038/nature11709 [PubMed: 23151479]

- Yamaguchi S, Kimura H, Tada M, Nakatsuji N, & Tada T (2005). Nanog expression in mouse germ cell development. *Gene Expression Patterns*, 5(5), 639–646. doi:10.1016/j.modgep.2005.03.001 [PubMed: 15939376]
- Yamaguchi S, Shen L, Liu Y, Sendler D, & Zhang Y (2013). Role of Tet1 in erasure of genomic imprinting. *Nature*, 504, 460–464. doi:10.1038/nature12805 [PubMed: 24291790]
- Yamaji M, Seki Y, Kurimoto K, Yabuta Y, Yuasa M, Shigeta M, ... Saitou M (2008). Critical function of Prdm14 for the establishment of the germ cell lineage in mice. *Nat Genet*, 40(8), 1016–1022. doi:10.1038/ng.186 [PubMed: 18622394]
- Yamashiro C, Sasaki K, Yabuta Y, Kojima Y, Nakamura T, Okamoto I, ... Saitou M (2018). Generation of human oogonia from induced pluripotent stem cells in vitro. *Science*. doi:10.1126/science.aat1674
- Yamashita K, Shinohara M, & Shinohara A (2004). Rad6-Bre1-mediated histone H2B ubiquitylation modulates the formation of double-strand breaks during meiosis. *Proc Natl Acad Sci U S A*, 101(31), 11380–11385. doi:10.1073/pnas.0400078101 [PubMed: 15280549]
- Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, ... Matzuk MM (2001). Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol*, 15(6), 854–866. doi:10.1210/mend.15.6.0662 [PubMed: 11376106]
- Yang QE, Kim D, Kaucher A, Oatley MJ, & Oatley JM (2013). CXCL12-CXCR4 signaling is required for the maintenance of mouse spermatogonial stem cells. *Journal of Cell Science*, 126(4), 1009–1020. doi:10.1242/jcs.119826 [PubMed: 23239029]
- Yao HH, DiNapoli L, & Capel B (2003). Meiotic germ cells antagonize mesonephric cell migration and testis cord formation in mouse gonads. *Development*, 130(24), 5895–5902. doi:10.1242/dev.00836 [PubMed: 14561636]
- Yao HH, Matzuk MM, Jorgez CJ, Menke DB, Page DC, Swain A, & Capel B (2004). Follistatin operates downstream of Wnt4 in mammalian ovary organogenesis. *Dev Dyn*, 230(2), 210–215. doi:10.1002/dvdy.20042 [PubMed: 15162500]
- Yartseva V, & Giraldez AJ (2015). The Maternal-to-Zygotic Transition During Vertebrate Development: A Model for Reprogramming. *Current Topics in Developmental Biology*, 113, 191–232. doi:10.1016/bs.ctdb.2015.07.020 [PubMed: 26358874]
- Ying Y, Liu XM, Marble A, Lawson KA, & Zhao GQ (2000). Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol Endocrinol*, 14(7), 1053–1063. doi:10.1210/mend.14.7.0479 [PubMed: 10894154]
- Ying Y, & Zhao GQ (2001). Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Dev Biol*, 232(2), 484–492. doi:10.1006/dbio.2001.0173 [PubMed: 11401407]
- Yoshida K, Kondoh G, Matsuda Y, Habu T, Nishimune Y, & Morita T (1998). The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. *Mol Cell*, 1(5), 707–718. [PubMed: 9660954]
- Yoshida S, Sukeno M, Nakagawa T, Ohbo K, Nagamatsu G, Suda T, & Nabeshima Y. i. (2006). The first round of mouse spermatogenesis is a distinctive program that lacks the self-renewing spermatogonia stage. *Development*, 133, 1495–1505. doi:10.1242/dev.02316 [PubMed: 16540512]
- Yoshida S, Takakura A, Ohbo K, Abe K, Wakabayashi J, Yamamoto M, ... Nabeshima YI (2004). Neurogenin3 delineates the earliest stages of spermatogenesis in the mouse testis. *Developmental Biology*, 269, 447–458. doi:10.1016/j.ydbio.2004.01.036 [PubMed: 15110712]
- Zelazowski MJ, Sandoval M, Paniker L, Hamilton HM, Han J, Gribbell MA, ... Cole F (2017). Age-Dependent Alterations in Meiotic Recombination Cause Chromosome Segregation Errors in Spermatocytes. *Cell*, 171(3), 601–614 e613. doi:10.1016/j.cell.2017.08.042 [PubMed: 28942922]
- Zeleznik AJ (2004). The physiology of follicle selection. *Reprod Biol Endocrinol*, 2, 31. doi:10.1186/1477-7827-2-31 [PubMed: 15200680]
- Zhang B, Zheng H, Huang B, Li W, Xiang Y, Peng X, ... Xie W (2016). Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. *Nature*, 537, 553–557. doi:10.1038/nature19361 [PubMed: 27626382]

- Zhang FP, Poutanen M, Wilbertz J, & Huhtaniemi I (2001). Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. *Mol Endocrinol*, 15(1), 172–183. doi:10.1210/mend.15.1.0582 [PubMed: 11145748]
- Zheng H, Huang B, Zhang B, Xiang Y, Du Z, Xu Q, ... Xie W (2016). Resetting Epigenetic Memory by Reprogramming of Histone Modifications in Mammals. *Molecular Cell*, 63, 1066–1079. doi:10.1016/j.molcel.2016.08.032 [PubMed: 27635762]
- Zhou Q, Nie R, Li Y, Friel P, Mitchell D, Hess RA, ... Griswold MD (2008). Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an in vivo study in vitamin A-sufficient postnatal murine testes. *Biol Reprod*, 79(1), 35–42. doi:10.1095/biolreprod.107.066795 [PubMed: 18322276]
- Zhou Q, Nie R, Prins GS, Saunders PTK, Katzenellenbogen BS, & Hess R. a. (2002). Localization of androgen and estrogen receptors in adult male mouse reproductive tract. *J Androl*, 23, 870–881. doi:10.1002/j.1939-4640.2002.tb02345.x [PubMed: 12399534]
- Zhou Q, Wang M, Yuan Y, Wang X, Fu R, Wan H, ... Zhou Q (2016). Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro. *Cell Stem Cell*, 18(3), 330–340. doi:10.1016/j.stem.2016.01.017 [PubMed: 26923202]
- Zhou X, Kudo a., Kawakami H, & Hirano H (1996). Immunohistochemical localization of androgen receptor in mouse testicular germ cells during fetal and postnatal development. *The Anatomical Record*, 245, 509–518. doi:10.1002/(SICI)1097-0185(199607)245:3<509::AID-AR7>3.0.CO;2-M [PubMed: 8800409]

Figure 1.

PGC specification in mice and humans during gastrulation. PGC specification in mice and humans during gastrulation. Adapted from Kobayashi, T., & Surani, M. A. (2018). On the origin of the human germline. *Development*, 145(16). doi:[10.1242/dev.150433](https://doi.org/10.1242/dev.150433).

Figure 2.

The process of oogenesis in mammals. The process of oogenesis in mammals. Adapted from Ikami, K., Nuzhat, N., & Lei, L. (2017). Organelle transport during mouse oocyte differentiation in germline cysts. *Current Opinion in Cell Biology*, 44, 14–19. doi:[10.1016/j.ceb.2016.12.002](https://doi.org/10.1016/j.ceb.2016.12.002).

Figure 3.

Fig. 3. (A) Schematic overview of testis cross section with major somatic and germ cell types shown, along with select paracrine and endocrine signaling molecules. (B) Progression of spermatogenesis. BTB, blood-testis barrier; FSH, follicle stimulating hormone; GDNF, Glial derived neutrophic factor; LH, luteinizing hormone; RA, Retinoic acid; T, testosterone.

Figure 4. Epigenetic dynamics during germ cell specification, gametogenesis, and early development.